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Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

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Comparative Evaluation of Morphological Findings and Flow-Sensitive Magnetic Resonance Parameters in Patients with Chiari 1 Malformation Against Healthy Individuals

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ABSTRACT

Introduction: A better understanding of the cerebrospinal fluid (CSF) flow dynamics of Chiari 1 malformation (CM-1) may help to reach more specific parameters for indicating therapy and response to therapy. This study developed a parameter to better characterize CSF flow alterations in the cerebral aqueduct in patients presenting with CM-1.

Methods: We retrospectively scanned archives for CM-1 patients who underwent CSF flow analysis between January 2016 and December 2022. Eighty-eight CM-1 patients and 83 control cases were included in the study. The cerebellar tonsillar descensus was measured in all patients. In 11 cases with the syrinx cavity, the largest transverse diameter of the cavity was measured. Phase-contrast magnetic resonance imaging was performed using a 1.5 T scanner. Peak velocity (P-Vel) (cm/s), average velocity (A-Vel) (cm/s), forward flow volume (µL), reverse flow volume (µL), peak flow (P-Fl) (mL/s), and time to peak velocity (TP-Vel) (s) and cerebral aqueduct's stroke volume (µL) were calculated. Sixteen CM-1 patients underwent decompression surgery, and we examined postoperative CSF flow analyses.

Results: A-Vel and P-F1 were statistically significantly higher in CM-1 cases than in controls (p=0.006, p=0.037). There was a significant negative correlation between the diameter of the syrinx cavity and P-Vel and P-Fl, and a positive correlation between TP-Vel. We found no significant difference between the postoperative and preoperative CSF flow parameters of the CM-1 patients.

Conclusion: P-Fl differs between the patient and control groups and correlates with the syrinx diameter; therefore, it could be a useful parameter in CSF flow analysis in CM-1 cases. However, higher case numbers can achieve more effective results.

Keywords: Chiari 1 malformation, CSF flow, PC-MRI, peak flow

Introduction

Chiari malformations (CM) are defined as varying degrees of extension of the brainstem and cerebellar structures into the cervical spinal canal (1). CM-1 is a congenital anomaly in which the cerebellar tonsils extend caudally and is the most common group among CMs (2). CM-1 cases may be asymptomatic and occur incidentally in magnetic resonance imaging (MRI) examinations, which are increasing nowadays (3,4). Symptoms ranging from a mild headache to difficulty swallowing and an unsteady gait, may also occur. If symptomatic, patients present clinical signs of brain stem compression or syringomyelia in adulthood (5). However, the incidence of clinically significant CM-1 anomalies is quite low (1,3). Syrinx cavities, hydrocephalus, and skeletal anomalies may accompany CM-1 cases at different rates (6,7).

The main purpose of surgery in CM-1 malformations is to restore cerebrospinal fluid (CSF) flow at the level of the foramen magnum and around the brain stem. The applied decompression surgery removes a part of the occipital bone and posterior arch of the first cervical vertebra (C1). Thus, it reduces the pressure at the craniocervical junction level and the width of the syrinx cavity (1). It has been shown that CM-1 patients benefit from decompression surgery. However, not all patients may benefit equally from this treatment. Therefore, it is necessary to select suitable patients for the operation (8,9).

A better understanding of the effects of CM-1 pathophysiology on CSF flow dynamics may lead to the identification of diagnostic tools that can more specifically predict the indication for therapy and the response to therapy. Therefore, this study developed a parameter to better characterize CSF flow alterations in the cerebral aqueduct in patients presenting with

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CM-1. In the literature, different studies have been conducted on CSF flow changes at the level of the craniocervical junction in patients with CH-1 (10-12). However, because the intracranial space is a closed area, dynamic changes in the outlet of the CSF may also be reflected in the flow dynamics at proximal CSF distances and cause flow disturbances. Because the normal anatomy of the craniocervical junction will be disrupted after decompression surgery, it will be difficult to make quantitative measurements at this level. Furthermore, velocity aliasing artifacts from the vertebral arteries can complicate phase-contrast magnetic resonance imaging (PC-MRI) flow measurements at the craniocervical junction. For these reasons, we measured and evaluated the cerebral aqueduct level instead of the craniocervical junction. First, we compared the dynamic CSF flow parameters in the cerebral aqueduct between CM-1 and normal cases. We also compared the postoperative and preoperative values of CSF flow dynamics at the cerebral aqueduct level in CM-1 patients who underwent decompression surgery.

Methods

Subjects

After obtaining the Ethics Committee approval from University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 20, date: 27.01.2023), our medical records were retrospectively scanned for patients who underwent CSF flow analysis between January 2016 and December 2022. The diagnosis of CM-1 can be made if the cerebellar tonsils extend 5 mm or more from the level of the foramen magnum to the caudal in adults (13). The study did not include cases with cerebellar tonsils extending 5 mm or less. Cases with intracranial hypertension (secondary or idiopathic) and intracranial hypotension were excluded from the study. Only cases aged 18 years and over were included in the study because there may be changes in clinical management. Patients whose CSF flow examinations had artifacts and inappropriate images for measurement were also excluded from the study. Finally, 88 cases diagnosed with CM-1 after excluding differential diagnoses were included in the study. Sixteen patients were operated on for CM-1 and had postoperative CSF flow examination. These images were obtained for follow-up on an average 3 to 6 months after the operation. Eighty-three patients who underwent CSF flow examination with headache in a similar time interval and had a stress-related cluster-tension headache diagnosis were also included in the study as the control group.

Image Acquisition and Analyses

All MRI were performed with a 1.5 T-scanner (Siemens Healthcare, Aera Magnetom, Erlangen, Germany) equipped with an 8-channel head coil. First, each case was evaluated with conventional MR sequences, including 3D T2-SPACE sequences (slice thickness: 1 mm, FOV: 240 mm, matrix 231×256, TR: 2500, and TR: 501). CSF flow imaging was performed using the two-dimensional phase-contrast MRI technique in the axial and midsagittal planes. Axial views were planned perpendicular to the extent of the cerebral aqueduct on the midsagittal view of PC-MRI. Rephase, magnitude, and phase images were obtained in the axial and sagittal planes. Cardiac triggering was retrospectively used for an average of 15-20 cardiac phases according to the heart rate. Velocity encoding (Venc) was determined as 5, 10, and 20 cm/s for each patient. Flow in the caudocranial direction (diastolic) was coded as positive, and the craniocaudal direction (systolic) was coded as negative.

MRI of all patients were transferred to the Syngo Via® workstation (Siemens Medical Solutions) for measurement with appropriate software. All images were examined separately by a radiologist experienced in neuroradiology and CSF flow examination for five years (Direnç Özlem Aksoy). The distance of the caudal endpoint of the cerebellar tonsils from the plane of the foramen magnum (the line drawn from the opisthion to the basion) was measured in sagittal SPACE images of each case (Figure 1A). In addition, the 11 cases with a syrinx cavity were identified, and the widest mediolateral diameter of the syrinx cavity was measured on the axial SPACE image (Figure 1B). Axial PC-MRI images were used for CSF flow quantification. Measurements were made on the image acquired at the appropriate Venc setting with the brightest flow signal without aliasing artifacts. A manual region of interest (ROI) indicated flow boundaries observed in the cerebral aqueduct (Figure 2). The ROI was also copied into all previous and subsequent phase images acquired during a cardiac cycle to obtain the time-velocity curve. Peak velocity (P-Vel) (cm/s), average velocity (A-Vel) (cm/s), forward flow volume (FV) (µL), reverse flow volume (RV) (µL), peak flow (P-Fl) (ml/s), and time to peak velocity (TP-Vel) (s) were calculated from time-velocity curve data automatically by the software (Figure 1). We manually calculated the cerebral aqueduct stroke volume (SV) (µL) as the average of FV and RV.

Statistical Analysis

In the descriptive statistics of the data, mean, standard deviation, median, minimum, maximum, frequency, and ratio values were used. The distribution of variables was measured using the Kolmogorov-Smirnov test. Independent sample t-test and Mann-Whitney U test were

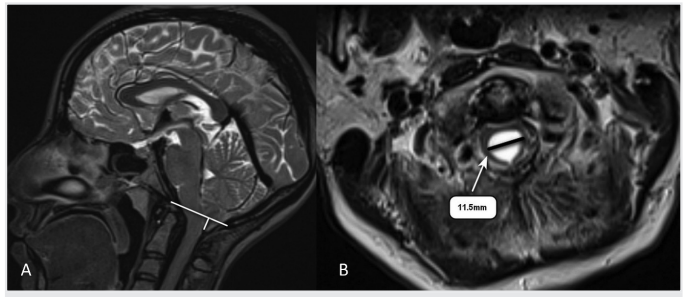

Figure 1. Measurement of the cerebellar tonsillar descensus in the sagittal SPACE image (A) and syrinx cavity in the axial SPACE image (B)

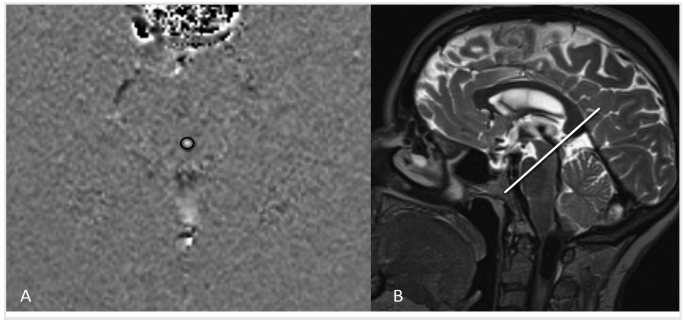

Figure 2. Aqueductal ROI placement in the axial phase image (A) and corresponding aqueduct level in the sagittal plane (B)

ROI: Region of interest

used to analyze independent quantitative data. Paired-sample t-test and Wilcoxon test were used to analyze dependent quantitative data. The chi-square test was used to analyze independent qualitative data, and the Fisher's exact test was used when the chi-square test conditions were not met. Spearman correlation analysis was used in the correlation analysis. SPSS 28.0 program was used in the analysis.

Results

There were 88-CM-1 cases and 83 control cases in our study. While the mean age was 47.3±18.5 in the control group, the mean age in CM-1 patients was 40.4±14.0 years. 65.1% (54) of the control group were female and 65.1% (29) were male. 76.1% (67) of CM-1 patients were female and 23.9% (21) were male. The results of the CSF flow measurements for CM-1 cases and the control group are summarized in Table 1. A-Vel and P-F1 were statistically significantly higher in CM-1 cases than controls (p=0.006, p=0.037). We found no significant difference between the patient and control groups in other parameters. The analysis we conducted to show the correlation of the distance of the caudal decensus of the cerebellar tonsils and the width of the syrinx cavity with the CSF flow parameters in CM-1 cases is also summarized in Table 2. There was no statistically significant correlation between the distance of the descensus and the flow parameters. There was a significant negative correlation between the diameter of the syrinx cavity and P-Vel and P-Fl, and a positive correlation between TP-Vel. We compared the postoperative CSF flow measurement parameters of 16 patients with CM-1 who underwent decompression surgery with their preoperative measurements (Table 3). We found no significant difference in the parameters between the two groups.

Table 1. CSF flow measurements for CM-1 and control group

	Control		CM-1		p	
	Mean ± SD/(n, %)	Median	Mean ± SD/(n, %)	Median		
P-Vel (cm/s)	-2.9±7.8	-6.0	-3.6±7.5	-6.6	0.471	[m]
A-Vel (cm/s)	0.13±0.27	0.13	0.20±0.18	0.20	**0.006**	[m]
TP-Vel (s)	592.3±188.7	608.8	597.1±193.2	624.7	0.667	[m]
FV (µL)	48.6±25.2	45.0	53.5±32.2	44.5	0.650	[m]
RV (µL)	41.0±23.9	35.0	40.7±28.0	34.5	0.524	[m]
SV (µL)	44.8±23.5	39.0	47.1±29.5	39.5	1.000	[m]
P-Fl (µL/s)	-26.3±243.7	-112.0	-100.2±240.4	-149.0	**0.037**	[m]

[m] Mann-Whitney U test. Bolded p-values are for statistically significant results (p<0.05). P-Vel: Peak velocity, A-Vel: Average velocity, TP-Vel: Time to peak velocity, FV: Forward volume, RV: Reverse volume, SV: Stroke volume, P-Fl: Peak flow, SD: Standard deviation, CSF: Cerebrospinal fluid, CM-1: Chiari 1 malformation

Table 2. Correlation analysis of CSF flow parameters with descensus distance and syrinx width in CM-1 cases

	Descensus (mm)		Syrinx (mm)	
	r	p	r	p
P-Vel (cm/s)	-0.029	0.786	-0.706	**0.015**
A-Vel (cm/s)	-0.010	0.927	-0.296	0.377
TP-Vel (s)	-0.048	0.660	0.606	**0.048**
FV (µL)	-0.006	0.954	0.524	0.098
RV (µL)	0.034	0.756	0.571	0.067
SV (µL)	0.025	0.818	0.524	0.098
P-Fl (µL/s)	-0.036	0.736	-0.715	**0.013**

Spearman correlation. Bolded p-values are for statistically significant results (p<0.05). P-Vel: Peak velocity, A-Vel: Average velocity, TP-Vel: Time to peak velocity, FV: Forward volume, RV: Reverse volume, SV: Stroke volume, P-Fl: Peak flow, CSF: Cerebrospinal fluid, CM-1: Chiari 1 malformation

Table 3. Postoperative and preoperative CSF flow measurement parameters of CM-1 patients undergoing decompression surgery

	Preoperative		Postoperative		p	
	Mean ± SD	Median	Mean ± SD	Median		
P-Vel (cm/s)	-3.7±8.0	-7.2	-5.8±6.5	-7.3	0.163	[w]
A-Vel (cm/s)	0.28±0.19	0.27	0.26±0.22	0.23	0.753	[p]
TP-Vel (s)	635.6±176.7	682.8	650.5±202.8	674.6	0.569	[w]
FV (µL)	65.3±28.7	73.0	57.2±30.9	47.0	0.326	[w]
RV (µL)	44.1±24.8	43.0	43.1±22.0	35.5	0.791	[p]
SV (µL)	54.7±25.3	56.0	50.1±26.0	40.5	0.386	[p]
P-Fl (µL/s)	-99.3±271.1	-181.0	-82.5±238.3	-157.0	0.469	[w]

[p]: Paired sample t-test, [w]: Wilcoxon test. Bolded p-values are for statistically significant results (p<0.05). P-Vel: Peak velocity, A-Vel: Average velocity, TP-Vel: Time to peak velocity, FV: Forward volume, RV: Reverse volume, SV: Stroke volume, P-Fl: Peak flow, CSF: Cerebrospinal fluid, CM-1: Chiari 1 malformation, SD: Standard deviation

Discussion

There is a cyclic flow of CSF in the craniocaudal direction in the systole phase of the cardiac cycle and the caudocranial direction in the diastole phase (14). On PC-MRI, the cephalad flow of CSF during diastole is positively coded above the flow time curve. The caudal flow of CSF, on the other hand, is negatively coded under the flow time curve during systole (15,16). PC-MRI is the only non-invasive method to assess the direction and amount of this biphasic CSF flow (17,18). Therefore, many studies have been conducted using PC-MRI in normal cases and diseases expected to make differences in CSF flow dynamics, such as normal pressure hydrocephaly, idiopathic intracranial hypertension, hydrocephaly, atrophy, and CM-1 (19-21).

As a result of obliteration at the level of the foramen magnum due to herniation of the cerebellar tonsils, CSF flow may be restricted, and higher velocities and turbulence may be produced in CM-1 (22). It has been argued that changes in CSF P-Vel values in the aqueduct of Sylvius are sensitive to detect changes caused by increased intracranial pressure (18). In our study, P-Vel and P-Fl in the caudal direction in the cerebral aqueduct were higher in CM-1 cases than in the control group. Although we could not find a statistically significant difference in P-Vel (p=0.471), our difference in P-Fl (p=0.037) was significant. In addition, A-Vel was significantly higher in CM-1 cases (p=0.006). Bapuraj et al. (23), in their study on a pediatric case group, found the mean velocity amplitude and P-Vel amplitude at the level of the cerebral aqueduct to be higher in patients with CM-1 malformation compared to the control group. Wang et al. (15), in their study comparing the caudal and cephalad flow separately, found that the P-Vel of the cephalad flow of the aqueduct in CM-1 patients was significantly lower than the control (p=0.022). The PV of the caudal flow was higher than that of healthy controls (p=0.004) (15). Ahmad et al. (21), in their study on different diseases, found that peak diastolic velocity (p=0.03) and peak systolic velocity (p=0.003) significantly higher in CM-1 cases than in the control group. In the study of Liu et al. (24), maximum velocity was found to be higher in both caudal (p=0.018) and cranial (p=0.007) directions in the patient group when compared to the control group. However, we did not detect any were significant difference in SV (p=1.000). In our study, SV values at the cerebral aqueduct level in CM-1 cases were slightly higher compared with the control group, but it was not statistically significant (p=1.000). Slightly high results for SV at the cerebral aqueduct of CM-1 patients were obtained in the literature, which was not statistically significant and similar to our results (p=0.06) (21).

This increase in CSF flow velocities could represent an etiologic factor for syrinx formation (22). CSF must flow to the caudal from the foramen magnum to regulate the intracranial pressure that rises with blood inflow to the brain during the systole phase of the cardiac cycle (25). Our study also evaluated whether tonsillar decensus and syrinx cavity width correlated with flow parameters. According to the data we obtained, we did not detect a significant correlation between the caudal descensus distance of the cerebellar tonsils and the flow data. However, in our comparison with the width of the syrinx cavity, we found a significant negative correlation between the syrinx cavity width and P-Vel (p=0.015) and P-Fl (p=0.013). In addition, as the diameter of the syrinx cavity increased, TP-Vel also increased (p=0.048). Apart from that, we did not find any significant relationship between syrinx and SV. Capel et al. (26) also found no significant difference (p=0.13) in SV in the cerebral aqueduct when they compared patients with and without syringomyelia in CM-1 patients, which was also correlated with our results.

We also compared the preoperative and postoperative CSF flow parameters of CM-1 cases. However, we could not find a statistically significant difference. Capel et al. (26) also did not detect a statistically significant difference in the SV of the aqueduct after surgery (p=0.36). In the study of Wang et al. (15), the P-Vel of the cephalad flow of the aqueduct in CM-1 patients increased after surgery (p=0.003), while the P-Vel of the caudal flow decreased (0.012) (15). On the other hand, Bapuraj et al. (23) detected a statistically significant decrease in the amplitude of mean velocity in postoperative cases. However, there was no significant change in the amplitude of peak velocity (23).

Study Limitations

Our study has some limitations. The retrospective nature and small number of patients are the main limitations. A single radiologist made the measurements, so the interobserver and intraobserver agreement was not evaluated. Since we did not consider which cases were clinically symptomatic and which were not, we could not make an evaluation and comparison in this direction. We did not categorize surgical procedures and could not evaluate whether the difference in surgical approach affected postoperative results. In addition, we could not evaluate the relationship between preoperative flow parameters and surgical outcomes.

Conclusion

MRI plays an important role in the diagnosis and follow-up of CM-1 cases. In addition, because it will not be possible to detect changes in CSF flow with conventional methods, methods that are more sensitive to dynamic changes, such as PC-MRI, can be used. Therefore, we studied the CSF flow changes that we can detect with PC-MRI in CM-1 cases. We found a significant difference in A-Vel (cm/s) (p=0.006) and P-Fl (µL/s) (p=0.037) in CM-1 cases compared with the control group. We also found a statistically significant correlation between syrinx cavity diameter and P-Vel (cm/s) (p=0.015), TP-Vel (s) (p=0.048), and P-Fl (µL/s) (p=0.013). Since P-Fl differs between the patient and control groups and correlates with the syrinx diameter, it could be a useful parameter in CSF flow analysis in CM-1 cases. Although no significant difference was found in postoperative cases in this parameter, higher case numbers can achieve more effective results.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (approval number: 20, date: 27.01.2023).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - V.G., A.S.M.; Design - D.Ö.A., S.K.G.; Data Collection or Processing - İ.Y., Y.K.; Analysis or Interpretation - D.Ö.A.; Literature Search - D.Ö.A., İ.Y., Y.K.; Writing - D.Ö.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Comparative Results of Surgical Treatment of Chronic Subdural Hematoma with Single and Double Burr Hole

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ABSTRACT

Introduction: Chronic subdural hematomas (CSDH) are intracranial hematomas that are usually seen in the middle and advanced age. They are seen as a result of bleeding from the parasagittal bridging veins. The results of patients who were surgically treated with single- and double-burr hole drainage due to CSDH in our clinic were investigated. It has been tried to decide which of these two methods is more suitable for surgical treatment.

Methods: We retrospectively reviewed 146 patients hospitalized with CSDH and treated with burrhole drainage in our clinic between 2011 and 2021. Informed consent forms were obtained from each patient. We divided the surgical treatments that we applied to the patients; into two groups: single burrhole without irrigation (group A, n=41) and double burrhole with irrigation (group B, n=105). The results were compared as radiological and clinical factors. The width of hematomas was determined by magnetic resonance imaging and defined as the maximal diameter in the coronal orientation perpendicular to the skull curvature. The thickness of inner membrane was measured on constructive interference steady state images. The imaging characteristics of hematomas on computed tomography, if available, were also reviewed and defined as hypodense, hyperdense, and inodense in comparison with cerebral paraenchyma.

Results: The change in subdural hematoma thickness was 68.38±10.10% in group A and 53.7%±31.9% in group B. The change in midline shift was 58.6%±24.5 in group A and 53.7%±31.9% in group B. There was no statistically significant difference in hematoma evacuation and recovery of midline shift between the two groups. Recurrence occurred in 5 (12.1%) cases in group A and 8 (7.6%) cases in group B. In terms of recurrence, both groups were similar.

Conclusion: Similar hematoma evacuation and midline shift improvement were observed between the two surgical techniques. We think that both methods have similar efficacy for treating CSDH.

Keywords: Subdural, burr hole, surgery, hematoma

Introduction

Chronic subdural hematomas (CSDH) are one of the most common types of intracranial hemorrhages and show a good prognosis when properly diagnosed and treated (1,2).

CSDH is an intracranial hemorrhage that is usually seen in middle and old age and develops as a result of minor head trauma. While its incidence is 3.4/100,000 before the age of 65, this rate increases to 8-58/100,000 after the age of 65. The most important etiological cause is bleeding due to trauma of the parasagittal bridging veins, which are stretched as a result of cerebral atrophy, which is age-related. Although most of these cases do not remember the trauma, trauma is included in the history of 60-80% of them (3,4).

Among the signs and symptoms, the most common symptom is headache, which usually occurs due to increased intracranial pressure. In addition, clinical findings that can be seen in CSDH include fainting, memory impairment, apathy, sleepiness, focal neurological deficit, and seizures (5).

Surgery is usually the chosen treatment for CSDH, and evacuation of the hematoma by burr hole or craniotomy is the most commonly preferred surgical method. The regression or disappearance of bleeding that is not surgically drained in CSDH is a rare condition, and the blood may remain calcified in the intracranial space (6).

In elderly and high-risk patients, hematoma drainage with a burr hole is preferred and is a less invasive method. However, the high recurrence rate of hematoma is a disadvantage of this surgical method (4). The choice of surgical method should be decided according to the tomographic finding of the hematoma, the clinical condition of the patient, and the age and presence of additional disease conditions (5).

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In this study, the surgical treatment of patientsdiagnosed with CSDH in our clinic with single and double burr holes and the clinical results are comparatively presented.

Methods

The study was approved by the Sakarya University Faculty of Medicine Ethics Committee (approval number: E-71522473-050.01.04-214486_07, date: 25.01.2023). Informed consent forms were obtained from each patient.

Case

Patients treated with burrhole drainage (BHD) with the diagnosis of CSDH between January 1, 2011 and February 1, 2021 in our clinic were retrospectively evaluated. The age of the patients, the size of the bleeding, and the additional diseases that could be detected were examined. A total of 155 patients with subdural hematoma bleeding underwent a surgical procedure. Because 9 of 155 patients underwent craniotomy, these patients were not included in the study. The remaining 146 patients were treated with BHD. The patients were divided into two groups according to the surgical technique performed: Group A (n=41) single BHD without irrigation, group B (n=105) double BHD with irrigation. The chosen surgical method was determined according to the surgeon's habits and preferences.

Surgical Procedure

Thirty-six (88%) patients with single BHD were under local anesthesia (LA) and sedation, 5 (12%) were under general anesthesia, and 83 (79%) patients with double BHD were under LA and sedation. and 26 of them were operated under general anesthesia. According to the side of the hematoma (right-left-bilateral): a burrhole hole was opened in the parietal bone; directly over the parietal eminence, in patients with single BHD without irrigation (group A), and in the frontal (in the mid pupillary line behind the hairline) and parietal bone in patients with double BHD with irrigation (group B). After the dura was coagulated as plus, it was opened with a scalpel no. 15. In group A, a 12 N silicone catheter was placed to drain the hematoma without irrigation. In group B, after irrigation with saline, a 12 N silicone catheter extending from the burrhole in the parietal bone to the frontal region was placed. In all patients, the catheters were connected to the drainage bag and fixed at the head level. The catheters were removed after it was concluded that the hematoma was sufficiently drained in the neurological status of the patients and in the computerized brain tomography (CCT). This period was between 24 and 48 h on average. Postoperative (within 24 h), 10th day and 2nd month CT scans were performed on all patients.

Clinical Factors

In comparison of group A and group B, age, gender, hypertension status, diabetes, chronic kidney or heart failure, smoking, and coagulopathy disorders were investigated. When the additional diseases of CSDH patients were examined, it was observed that hypertension in 47 (44%) patients, diabetes mellitus type 2 in 34 (23%) patients, coagulopathy in 16 (10%) patients, chronic renal failure in 13 (12%) patients, and smoking in 39 (37%) patients were found to have a history. To compare the clinical outcomes of the two groups, the length of the hospital stay, mortality, and recurrence rates were evaluated.

Radiological Factors

Radiological parameters of the patients were evaluated preoperatively, postoperative first 24 h, early postoperative 10th day, and late postoperative 2nd month CBT. The thickness of the subdural hematoma and the length of the midline shift were evaluated preoperatively, early, and late postoperatively. The changes consist of percentages obtained by subtracting the postoperative values from the pre-operative values and dividing the pre-operative values (Figure 1, 2).

Statistical Analysis

Before data analysis, the normality of all variables was evaluated. For parametric variables, to summarize patient data, means and frequencies were used for continuous and categorical variables, respectively. If required, non-parametric counterparts of parametric tests were used. To test differences between means, independent samples t-test and to compare frequencies, chi-square test was used. The alpha value was set at 0.05. SPSS version 25 (IBM) was used (Table 1-3).

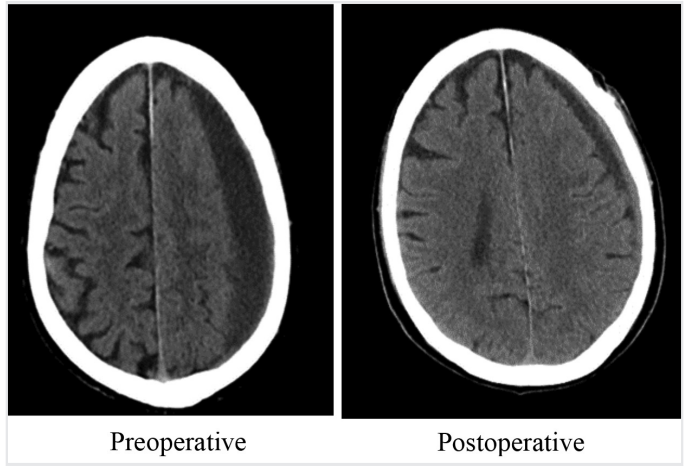

Figure 1. Double burr hole

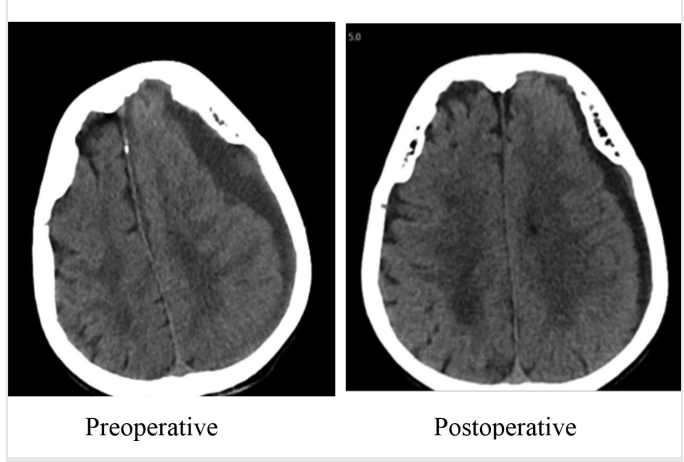

Figure 2. Single burr hole

Table 1. The summary of baseline demographics of patients with chronic subdural hematoma			
	Single burr-hole, (n=41)	**Double burr hole, (n=105)**	**p**
Age	72.71±1.50	72.02±1.68	0.790
Sex			
Male	10 (24.4%)	84 (80%)	**<0.001***
Female	31 (65.6%)	21 (20%)	
Comorbidities			
Hypertension	13 (31.7%)	34 (32.4%)	0.813
Diabetes mellitus	7 (17.1%)	27 (25.7%)	
Coagulopathy	4 (9.8%)	12 (11.4%)	
CKD	3 (7.3%)	14 (13.3%)	
Tobacco use	9 (22%)	30 (28.6%)	0.416
*Statistically significant (p-value <0.05). CKD: Chronic kidney disease			

Table 2. Pre- and postoperative radiological outcomes			
	Single burr-hole (n=41)	**Double burr-hole (n=105)**	**p**
Midline shift			
Pre	0.97±0.33	0.99±0.37	0.803
Early	0.72±0.25	0.77±0.34	0.448
Late	0.40±0.26	0.41±0.21	0.950
(%) change	58.6±24.5	53.7±31.9	0.430
Thickness			
Pre	2.33±0.52	2.62±0.76	0.029*
Early	1.32±0.30	1.40±0.46	0.269
Late	0.72±0.21	0.84±0.93	0.450
(%) change	68.38±10.10	62.90±62.83	0.621
*Statistically significant (p-value <0.05)			

Table 3. Recurrences and mortality rates			
	Single burr-hole (n=41)	**Double burr-hole (n=105)**	**p-value**
Recurrence (n, %)	5 (12.1%)	8 (7.6%)	0.382
Death	1 (2.4%) 1 (2.4%)	6 (5.71%) 1 (0.95%) 2 (3.81%) 1 (0.95%) 1 (0.95%) 1 (0.95%)	0.405
COVID-19			
Pneumonia			
Pulmonary embolism			
Heart failure			
Intracerebral hematoma			
COVID-19: Coronavirus disease-2019			

Results

Baseline demographic characteristics of patient data are summarized in Table 1. The total number of patients was 41 (28%) of patients were operated with a single burr-hole and 105 (72%) of them were operated with a double burr-hole approach. Mean age of single and double burrhole groups were not different (72.71 vs. 72.02, p=0.790). Sex distribution was not balanced, and the majority of the patients were females (65.6%) for single and males (80%) for double burr-hole groups. The comorbidities of patient groups were comparable and did not show any statistical difference (p=0.813).

With regard to radiological parameters, midline shift and hematoma thickness were calculated. Midline shift in the pre-operative period was equal for both groups and the percent change in midline shift did not differ between two different burrhole approaches (58.6 vs. 53.7, p=0.430). Thickness of subdural hematoma was higher in the double burr-hole group (p=0.029), but the percent change in thickness measured from pre-operative to late postoperative period did not show any statistical difference (p=0.621).

Recurrences occurred in 12.1% (n=5) and 7.6% (n=8) of all cases for single and double burrhole groups, respectively. Both groups were similar with

regard to recurrence rates (p=0.382). In the single burr hole group, only one patient died from coronavirus disease-2019 (COVID-19) infection (2.4%), whereas in the double burr hole group, there were 6 deaths (5.71%) caused by COVID-19 infection (n=1), pulmonary embolism (n=1), heart failure (n=1) and pneumonia (n=2). Another patient from the double burr-hole group died because of an intracerebral hematoma during the early postoperative period. Two groups did not differ with regard to mortality rates (2.4% vs 5.71%, p-value=0.405). Summary data on recurrences and mortality rates can be found in Table 3.

Discussion

CSDH is a type of intracerebral hematoma that is frequently seen in elderly patients with a systemic disease and is caused either because of a head trauma or spontaneously (5,6). In cases with previous cerebral trauma in the etiology of chronic subdural hemorrhage clinical findings are usually seen in almost 20 days and afterwards (7).

CSDH is bleeding between the inner and outer layers of the dura caused by recurrent multifocal hematomas of fragile sinusoidal vessels in the outer layer of the dura. It is a disease with low mortality and morbidity and generally good treatment results (5,8).

In the literature, it has been reported that the mean age of incidence is between 56 and 63 years, and 80% of the cases are above 50 years old (9,10).

Because most of the cases are elderly, their development on an atrophic brain background causes the clinical findings to appear more silently (11).

They reported that the most common presenting symptoms in patients with CSDH under 40 years of age are headache, nausea, and vomiting, but focal neurological findings constitute the most common complaint in patients over 75 years of age (12).

In cases of CSDH, the first radiological examination should be computed tomography of the brain (CCT). CCT should be preferred because it can show the bleeding, the shift caused by the bleeding, the probable duration of the bleeding, and it can be accessed quickly. In CCT, bleeding can be observed as hypodense, inodense, or hyperdense according to the time spent (13). Although CCT is usually the first choice as a diagnostic test in cases of CSDH, it has been reported that magnetic resonance imaging is beneficial for patients with recurrent bleeding at different stages, in cases with underlying tumor formation, and in cases where the duration of bleeding cannot be differentiated (14).

In CSDH, surgical evacuation of the hemorrhage should generally be the treatment modality. Surgical treatment options include twist drill craniotomy, craniotomy, and hematoma drainage with burr hole. Craniotomy was the preferred surgical approach, especially in periods when imaging methods were not very developed. In particular, this approach has been used in the foreground because it creates a wide area of intervention for the surgeon. However, the use of craniotomy in the surgical treatment of patients with CSDH has gradually decreased due to the long duration of the surgery and high blood loss. Still, it is applied especially in cases with thick membranes, in cases where brain expansion is incomplete and recurrent with burr hole drainage, and in bleedings where the hematoma is calcified (15).

Generally, in current surgical approaches to CSDH, it is recommended to perform bleeding drainage with a single or double burr hole, especially in cases with liquefied blood and without membrane and calcified hematoma. Bleeding drainage with a burr hole is a surgical method that is easier and has a low complication rate (16,17).

When studies in the literature are examined, the superiority of hematoma drainage with a single or double burr hole has not been proven. No matter which surgical method is preferred, it has been reported that the use of closed system drainage reduces recurrence. Surgical procedures with burr holes are preferred in advanced elderly patients with a high risk of mortality because they do not require anesthesia and can be performed at the bedside (18-21).

Old age, poor performance at presentation, cerebral atrophy, large hematoma, alcohol or anticoagulant use, renal failure, liver dysfunction, septum formation, or multiple membrane formation in the hematoma space are important risk factors for hematoma recurrence (12). Intraoperative or postoperative inadequate drainage and air collection in the hematoma space during surgery also increase the risk of recurrence (4).

The most common complication after surgical treatment for patients with CSDH is the recurrence of bleeding. It has been reported that the most important reason for this situation is the incomplete resection of the membrane and the lack of expansion of the atrophic brain (12). Another important problem is that the rapid evacuation of the hematoma during surgery may cause a sudden decrease in the intracranial pressure, causing brain shifts and acute hemorrhages in the opposite hemisphere (22).

Among the patients who were operated in our clinic, 2 patients had postoperative early rebleeding complications. These 2 patients underwent early craniotomy.

One of the common complications after surgery is tension pneumocephalus, which is seen at a rate of 0-10% (23). Another complication is postoperative epilepsy, reported at a rate of 2-19% (24). For the latter, the use of prophylactic antiepileptics for 6 months after the diagnosis of CSDH is recommended. Other complications that are observed after surgery are intracranial hypotension, subdural empyema, and intracranial acute hematoma (17).

No cases of pneumocephalus or early epilepsy were encountered in patients operated in our clinic. The patients were given prophylactic antiepileptic treatment for 3-6 months postoperatively.

The results were evaluated statistically. In the study, no difference was found in terms of subdural hematoma diameter in both preoperative and postoperative early and late periods between patients who underwent unilateral and bilateral procedures (p>0.05 for each).

Study Limitations

This study has potential limitations. There is very little prior research on this topic. More studies should be conducted on this method and contribution to the literature should be made.

Conclusion

Regarding the results of the surgical treatment performed with a single burr hole and the ones performed with a double burr hole in our clinic, both showed clinically significant improvement and a significant variability was statistically observed because of the patientstreatment. In addition, when single burr hole and double burr hole surgical treatments were compared, there were no statistically significant difference between them. However, the surgical method should be decided by considering the patient's age, clinic, history of anticoagulant use, and imaging methods.

Ethics Committee Approval: The study was approved by the Sakarya University Faculty of Medicine Ethics Committee (approval number: E-71522473-050.01.04-214486_07, date: 25.01.2023).

Informed Consent: Informed consent forms were obtained from each patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - S.H.; Concept - D.C.; Design - D.C.; Data Collection or Processing - M.K.; Analysis or Interpretation - T.K.; Literature Search - M.K.; Writing - T.K.

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Characteristics of Lung Patients Diagnosed with COVID-19 Pneumonia in the Intensive Care Unit and Their Effects on Prognosis

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ABSTRACT

Introduction: There is no clear evidence of an increased risk of coronavirus disease-2019 (COVID-19) infection in chronic obstructive pulmonary disease (COPD) and asthma. However, COPD patients diagnosed with COVID-19 have a high risk of mortality. No significant effects of COVID-19 have been demonstrated on mortality in asthma patients. In the present study, the purpose was to retrospectively examine the effects of the asthma and COPD diagnosis of patients hospitalized in the intensive care unit (ICU) with the diagnosis of COVID-19 pneumonia on prognosis.

Methods: The study was designed in a retrospective and descriptive design. Among the patients who were diagnosed with COVID-19 pneumonia and hospitalized in our ICU between March 11, 2020-January 31, 2021, all patients who had a history of COPD and/or asthma were included in the study. Invasive mechanical ventilation (IMV) durations, ICU stays, and 28-day mortality rates of the patients were recorded from the hospital information system.

Results: A total of 276 intensive care patients diagnosed with COVID-19 were examined. The frequency of the presence of COPD or asthma was determined as 8.69% (n=24) and 4.35% (n=12), respectively. Although IMV was applied to 25% (n=6) of COPD patients at the time of admission to the ICU, IMV was not applied to any of the asthmatic patients. The duration of hospitalization was 15 (9-30) days in COPD patients and 15 (13-24) days in asthma patients. Mortality was 75% (n=18) in patients with COPD and 25% (n=3) in patients with asthma.

Conclusion: In this retrospective analysis, the incidence of asthma and COPD in patients with COVID-19 was found to be similar to the literature data. The length of stay in the ICU was similar in asthma and COPD patients and was longer compared to other studies. COPD patients with COVID-19 pneumonia had higher IMV and mortality rates than asthma patients.

Keywords: COVID-19, chronic obstructive pulmonary disease, asthma, mortality rate

Introduction

Coronavirus disease-2019 (COVID-19) pandemic appeared in Wuhan, China toward the end of 2019. The novel type of coronavirus that caused the pandemic was named severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (1). It attaches to type 2 pneumocytes through the angiotensin converting enzyme-2 (ACE-2) receptors, enters the cells and creates infection after viral replication (2). COVID-19 pneumonia causes hyperinflammation and hypercoagulability and resulting in acute respiratory distress syndrome (3). It was reported in previous studies that it also causes approximately 4.5 million mortality worldwide, and this number is increasing with each passing day (4). It was shown that there is a relationship with clinical outcomes such as length of hospital stay, duration of mechanical ventilation, and mortality and patient characteristics such as age, gender, and comorbidities in the COVID-19 pandemic (5).

Chronic obstructive pulmonary disease (COPD) and asthma cause respiratory dysfunction by impairing lung functions and gas exchange. It is already known that asthma and COPD are common lung diseases in our society (6). Inter-country differences in the incidence of COVID-19 in these lung diseases were reported in previous studies, which also showed that the rates of COPD patients who contracted COVID-19 ranged from 2.0% to 17.7%. In their study, Leung et al. (7) reported that the release of ACE-2 is also high in COPD patients. It was also reported that both severe respiratory failure symptoms were seen more frequently and the mortality rate was higher in COVID-19 patients with COPD, while a systematic review and meta-analysis conducted in asthmatic patients

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reported that it did not cause increased mortality rates (7,8). Although it is known that asthma and COPD increase mortality in intensive care units (ICU), there are a limited number of intensive care studies on how COVID-19 progresses in asthma and COPD patients (7,9). In the present study, the purpose was to examine patients with COPD and asthma, which were reported to cause increased mortality in the ICU in patients hospitalized in the ICU with the diagnosis of COVID-19 pneumonia.

Methods

The study was approved by the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Clinical Research Ethics Committee (approval number: 2023/06-18, date: 13.07.2023).

The study had a retrospective and descriptive design. Among the patients who were diagnosed with COVID-19 pneumonia hospitalized in our ICU between March 11, 2020-January 31, 2021, all patients who had a history of COPD and/or asthma were included in the study, which was conducted in accordance with the Declaration of Helsinki Principles. Patients who were suggestive of COVID-19 both clinically and in imaging and who had positive real-time polymerase chain reaction test results were included in the study. Those with a diagnosis of malignancy and younger than 18 years were excluded from the study. Demographic data of the patients, clinical findings, laboratory parameters, Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, data on non-invasive mechanical ventilation (IMV)/IMV applications, drug treatments, length of stay in ICU, and 28-day mortality rates were recorded from the hospital data system.

Statistical Analysis

The data were analyzed with the SPSS 22.0 statistical package (SPSS, USA) and were presented as number of cases, percentage, or median (median-minimum and maximum). A p-value <0.05 was considered statistically significant.

Results

A total of 276 patients with COVID-19 pneumonia were reviewed for the study, and 28 patients were found to have a history of COPD and asthma. Three patients had an additional diagnosis of malignancy, and one patient was younger than 18 years. These 4 patients were excluded from the study, and 24 patients were included. A total of 24 (8.69%) of the patients had COPD and 12 (4.35%) had asthma (Table 1). When the patient's age was evaluated, the median age of patients with COPD was 77 (73-80), and the age of patients with asthma was 68.5 (57-75). Among the patients diagnosed with COPD, 19 (79.1%) were male and 5 (20.9%) were female. Two (16.7%) patients with asthma were male and 10 (83.3%) were female. The APACHE II score of asthma patients was 16.0 (10-22) and 16.5 (11-22) in COPD patients. The time elapsed between the onset of symptoms and admission to the ICU was recorded as 6 (3-8) days in COPD patients and 9 (8-15) days in asthma patients. The patients were also evaluated in terms of accompanying chronic diseases. In COPD patients, hypertension was detected in 13 (54%), diabetes mellitus (DM) in 8 (33%), heart disease in 12 (50%), cancer in 1 (4%), chronic liver disease in 3 (12%), and chronic kidney disease in 1 (4%) patient. In asthma patients, hypertension was detected in 7 (58%), DM in 4 (33%), heart disease in 1 (8%), cancer in 1 (8%), chronic liver disease in 1 (8%), and chronic kidney disease in 0 (0%) patients. Hypertension was the most common comorbidity in both COPD and asthma patients. The second most common comorbidity was heart disease in COPD patients, a and DM patients with asthama. The duration of hospitalization was 15 (9-30) days in COPD patients and 15 (13-24) days in asthma patients. Regarding the respiratory support treatments of COPD patients admitted to the ICU, 16 (66%) received oxygen only, 2 (8%) received NIV or high-flow oxygen treatment, and 6 (25%) received IMV treatment. Similarly, when respiratory support treatments of asthma patients were evaluated during hospitalization, there were 10 (83%) patients who received oxygen only, 2 (16%) patients who received NIV or high-flow oxygen treatment, and 0 (0%) patients who received IMV treatment. When the IMV treatments received by the patients during admission to the ICU were evaluated, there were 6 (25%) COPD patients and no patients who received IMV during admission with asthma. When re-evaluated on the 7th day in terms of the IMV treatments received, it was found that 19 (82%) of the hospitalized patients received IMV treatment. Although this rate was 15 (83%) in COPD patients, it was found in 4 (80%) patients with asthma (Table 1).

When the medical treatments received for COVID-19 were evaluated, the frequency of medical treatment in patients with COPD was favipiravir (Toyama Chemical, Japan) in 20 (83%) patients, steroid (Dexamethasone-Decort, Deva Holding, Turkey) in 16 (66%) patients, convalescent plasma in 11 (45%) patients, and tocilizumab (Hoffmann-La Roche and Chugai, Japan) in 3 (12%) patients. The frequency of medical treatment in asthmatic patients was favipravir in 10 (83%) patients, steroid in 8 (66%) patients, convalescent plasma in 6 (50%) patients, and tocilizumab in 1 (8%) patient (Table 1).

When 28-day mortality was evaluated in both groups, mortality was 75% (n=18) in patients with COPD and 25% (n=3) in patients with asthma (Table 1).

The laboratory parameters of the patients during hospitalization are given in Table 2. C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer and troponin values, which are widely accepted as mortality predictors for COVID-19, were higher in the COPD patient group (Table 2).

Discussion

This retrospective descriptive study was conducted on patients with COPD and asthma who were admitted to the ICU because of COVID-19 and obtained two important results. The first important point was that the incidence of COPD and asthma among patients with COVID-19 was substantial and similar to other studies in the literature. In studies conducted in Italy and New York including hospitalized patients, it was reported that the incidence of COPD varied between 2.4% and 14% in patients with a diagnosis of COVID-19 (7,9) The frequency of asthma shows significant differences between countries. It was shown that this rate was approximately 1-1.5% in China, and 5-6% in Spain, Brazil, Israel and Switzerland (10). Another remarkable point was the high mortality rate in COPD patients in our cohort. In another retrospective study that included hospitalized patients similar to our study, the mortality of patients with COVID + COPD was reported as 46.34% (11).

Table 1. General characteristics of the patients

	COPD and asthmatic patients (n=36)	Patients with asthama (n=12)	Patients with COPD (n=24)
Age, year	75 (69-79)	68.5 (57-75)	77 (73-80)
Gender (female/male)	15 (23.8%)/21 (86.2%)	10 (83.3%)/2 (16.7%)	5 (20.9%)/19 (79.1%)
APACHE II	16.50 (11-22)	16 (10-22)	16.5 (11-22)
The number of days between symptom onset and admission to intensive care unit	7 (5-10)	9 (8-15)	6 (3-8)
Concomitant chronic diseases			
Hypertension	20 (55%)	7 (58)	13 (54)
Diabetes mellitus	12 (33%)	4 (33)	8 (33)
Chronic heart disease	13 (36%)	1 (8)	12 (50)
Cancer	2 (5%)	1 (8)	1 (4)
Chronic liver disease	4 (11%)	1 (8)	3 (12)
Chronic kidney disease	12)	0 (0)	1 (4)
Respiratory support treatment on admission (n, %)			
Oxygen only	26 (72%)	10 (83)	16 (66)
NIMV and/or HFNC	4 (11%)	2 (16)	2 (8)
IMV	6 (16%)	0 (0)	6 (25)
Other treatments			
Favipiravir	30 (83%)	10 (83)	20 (83)
Steroid	24 (66%)	8 (66)	16 (66)
Convalescent plasma	17 (47%)	6 (50)	11 (45)
Tocilizumab	4 (11%)	1 (8)	3 (12)
Patients who received IMV on the 7th day in the intensive care unit [n=23, (%)]	19 (82%)	4 (80)	15 (83)
28-day mortality			
Yes/no (%)	21/15 (58%)	3 (25)	18 (75)
The length of the stay in intensive care unit (days)	12 (8-20)		
The length of stay in hospital (days)	15 (10-30)	15 (13-24)	15 (9-30)

COPD: Chronic obstructive pulmonary disease, APACHE II: Acute Physiology and Chronic Health Evaluation II, NIMV: Non-invasive mechanical ventilation, HFNC: High-flow nasal cannula, IMV: Invasive mechanical ventilation

Table 2. Laboratory values of the patients at admission

	All patients (n=36)	Patients with asthama (n=12)	Patients with COPD (n=24)
Hemoglobin (g/dL)	11.9 (10.4-13.3)	11.8 (10.5-12.2)	12.2 (10.4-14.4)
Neutrophil (/µL) $\times 10^9$	8.5 (5.8-11.0)	7.05 (3.6-9.2)	9.2 (6.3-12.7)
Lymphocyte (/µL) $\times 10^9$	0.50 (0.40-0.70)	0.5 (0.5-0.7)	0.5 (0.32-0.70)
Creatinine (mg/dL)	1.2 (1.0-1.7)	1.0 (0.6-1.2)	1.5 (1.0-2.0)
C-reactive protein (mg/L)	163 (97-221)	118 (72-210)	132 (68-199)
Procalcitonin (ng/mL)	0.19 (0.11-0.26)	0.22 (0.16-0.26)	0.15 (0.11-0.26)
Lactate dehydrogenase (U/L)	518 (341-781)	513 (347-537)	628 (302-827)
D-dimer (µg/L)	1540 (640-3710)	1030 (522(1825)	1830 (830-4170)
Troponin (ng/L)	22 (8-164)	9.5 (7-23)	89 (10-674)
Blood lactate level (mmol/L)	1.4 (1.1-2.1)	1.5 (1.1-1.8)	1.3 (1.1-2.4)
pO_2 (mmHg)	66 (47-75)	53 (45-66)	68 (47-80)
pCO_2 (mmHg)	41 (32-48)	43 (38-51)	39 (31-45)
pH	7.38 (7.28-7.47)	7.48 (7.34-7.49)	7.36 (7.25-7.40)

COPD: Chronic obstructive pulmonary disease, pO_2: Partial oxygen pressure, pCO_2: Partial carbon dioxide pressure

Although COVID-19 causes approximately 3.4 percent mortality worldwide, mortality is higher in critically ill patients in need of an ICU (12,13). In a systemic review and meta-analysis of Alqahtani et al. (14), it was reported that patients with COPD diagnosis as a comorbidity had more severe COVID-19 infection and mortality rates were higher than those without COVID-19. It is also emphasized that high mortality may occur because of vascular damage and thrombosis in COPD patients (13). SARS-CoV-2 uses ACE-2 as a receptor for intracellular entry (2). It is already known that the excretion of ACE-2, which is used as a receptor, is high in COPD patients, and although this is considered to be one of the reasons for the high mortality rate, it has not yet been proven (8). It was shown that COVID-19 does not cause increased mortality in patients with asthma (9).

In their meta-analysis, Fang et al. (15) reported that although the frequency of COPD among those with COVID-19 was between 2.0% and 17.7% (average around 2-4%), it was found to be higher at 8.6% in our patient cohort. This can be explained by the fact that our hospital was declared as a pandemic hospital (ward and intensive care) since the early period of the pandemic and that there was a chest diseases center nearby. Although it is a matter of debate why the prevalence of COPD was low in the current pandemic, it was emphasized that it may be because of the disease itself or its treatment modality. However, these remained at the level of hypotheses and could not be proven (13). In previous studies conducted with hospitalized patients with serious diseases, the frequency of asthma was shown to vary between 0.9% and 9% (16,17), but the frequency of asthma was found to be 4.3% in our cohort, which is consistent with the literature data.

The time between symptom onset and ICU admission was found to be consistent with the mean time (6-12 days) reported in the literature in COPD patients (18). Our rate of IMV application during admission to the ICU was found to be higher compared to the literature data (18). This may be because of ICU admission during the period when patients deteriorated to the point of intubation because of limited ICU beds. There was no patient who needed IMV at the time of ICU admission in our asthma patients.

It was reported in a systematic review that the length of stay in COPD patients was 8 days (19). In the present study, the reason why the hospital stay was longer in both asthma and COPD patients compared to the literature data can be explained by the admission of patients requiring ICU admission during the period when they deteriorated to the point of intubation because of limited ICU beds. A significant rate of our cohort had additional chronic diseases. We think that this may be a reason for the longer length of stay.

Hypertension, DM, and chronic heart disease were the most common comorbidities in both patient groups. In the literature, it has been shown that these additional diseases frequently accompany COVID-19 (5). The prevalence of chronic heart disease may be one of the reasons for the high mortality rate, especially in patients with COPD.

When the additional treatments applied because of COVID-19 were evaluated, it was seen that the favipravir treatment was used in almost all patients. This can be explained by the direct inclusion of favipravir treatment in the COVID-19 Treatment Guideline of the Ministry of Health until recently. The small cohort group that did not receive this treatment was in the period before favipravir entered the treatment guideline.

The second most common treatment seems to be steroids. It is seen that approximately two-thirds of the patients are administered steroids. The RECOVERY Collaborative Group conducted a randomized controlled trial of dexamethasone in hospitalized patients with COVID-19 and reported that 28-day mortality was lower in the dexamethasone group than in the usual care group (20). After their study, dexamethasone (or equivalent methylprednisolone) was included in the treatment guide of the Ministry of Health. This treatment was not given to one-third of patients because some of the patient cohort coincided with the period before the study of the RECOVERY Collaborative Group.

Convalescent plasma treatment was used as a part of treatment in many studies and was recommended in the treatment guide of the Ministry of Health in the early period of the COVID-19 pandemic. Convalescent plasma treatment was used in approximately half of our patients in both cohorts (21,22). In the current situation, the frequency of use has decreased significantly because of the debate about its effectiveness and the fact that it is not recommended to be given outside of a certain period (i.e., the first 5-7 days of the disease).

Low lymphocyte, CRP, troponin, LDH, and D-dimer elevation are shown to be predictors of poor prognosis in COVID-19 patients. Laboratory data were also found to be compatible with the literature in this study cohort. The current situation develops secondary to the inflammation caused by COVID-19 (17,19). Ferritin, which is also considered as an acute phase reactant, was shown to increase in COVID-19 infection and its height will be evaluated as a predictor of mortality (17). In the present study, ferritin values were not studied in the majority of our patients at admission to the ICU, and therefore, they could not be presented as data.

Study Limitations

The limitations of the study were that it had a retrospective and single-center design. However, although it was single-center, it included the evaluation of 276 tertiary intensive care patients.

Conclusion

The frequency of COPD and asthma is high in patients hospitalized in ICU because of COVID-19. High mortality and IMV rates were found in patients with COPD during intensive care. A closer follow-up of these patient groups is important. Further studies are needed to elucidate the etiology of poor prognosis in COPD patients.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Clinical Research Ethics Committee (approval number: 2023/06-18, date: 13.07.2023).

Informed Consent: Retrospective study.

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Analysis of Patients Resected for Primary Mediastinal Mass: Which Surgical Approach is Superior

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ABSTRACT

Introduction: In recent years, robot-assisted thoracoscopic surgery (RATS) and video-assisted thoracoscopic surgery (VATS) has been more frequently preferred in the surgical treatment of mediastinal masses. The number of studies comparing VATS with RATS is limited. In our study comparing the surgical outcomes of RATS and VATS procedures, we tried to determine the ideal treatment method.

Methods: Between 2016 and 2022, fifty-two patients who underwent minimally invasive surgical resection (VATS or RATS) for mediastinal mass were retrospectively analyzed.

Results: Mediastinal mass resection was performed by RATS (n=29) or VATS (n=23). 57.7% (n=30) of the mediastial masses were localized in the anterior mediastinum. The most common postoperative pathology was thymoma (27%, n=14). There was no surgical mortality. Grade 1 and 2 complications developed in 6 (11.5%) patients according to the Clavien-Dindo classification. Conversion to open surgery was required in a total of 5 patients [VATS group (n=3), 13% versus RATS group (n=2), 6.9%, p=0.644]. The median length of hospital stay was five days [VATS; 4 days interquartile range (IQR): 3-6] versus RATS; 5.5 days (IQR: 4-8), p=0.081]. The median drainage time was four days [VATS; 3 (2-5) versus RATS; 4.5 (3-7), p=0.133], and the mean drainage amount was 110 mL (70-190) (p=0.162). There was no significant difference between the duration of the operation (for VATS; 75.7±18.4 min, for RATS; 73.5±18.0 min, p=0.674). Postoperative pain scores were similar [median 2.19 (1-3) for RATS and 2.20 (1-3) for VATS, p=1.00].

Conclusion: RATS and VATS are reliable procedures offering many advantages in treating mediastinal masses. Both procedures have similar results in terms of the complication rate, the length of the hospital stay, and duration of surgery.

Keywords: Mediastinal mass, thoracoscopic surgery, thymectomy

Introduction

Mediastinal surgery presents many challenges due to the anatomical structure of the mediastinum and the large number of vital organs and tissues contained in it. Primary mediastinal masses include benign or malignant thymic tumors, neurogenic tumors, benign cysts, and germ cell tumors (1). Surgical resection of primary mediastinal masses is the gold standard treatment approach (2). As much as possible, minimally invasive surgical procedures should be preferred for resection of mediastinal masses (3).

Compared to open surgery, VATS offers many advantages, such as shorter operation time, lower complication rate, rapid postoperative recovery, minimal trauma, and better cosmetic appearance (4,5). Another method, RATS, has recently become an increasingly preferred minimally invasive surgical approach (6). Due to features such as high maneuverability, 3D visualization, and filtering hand tremor and not transferring it to the instrument, it allows tumor surgery in a narrow area such as the mediastinum to be performed safely and comfortably (1). Although there are many studies in the literature comparing minimally invasive surgical methods with open surgical approaches in treating mediastinal masses, the number of studies comparing VATS with RATS is limited (7).

Although there is a general acceptance that RATS and VATS are the first choice for resection of mediastial masses, there is no consensus on which procedure should be preferred. In our current study, we evaluated the surgical results of VATS and RATS and tried to determine the ideal of these minimally invasive surgical procedures.

Methods

The ethics committee approval of this study was obtained from the Ethics Committee of Sakarya University Faculty of Medicine Non-Interventional Ethics Committee and was conducted following the principles of the Declaration of Helsinki (approval number: E-71522473-050.01.04-216185-09, date: 31.01.2023).

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The files of 52 patients who underwent minimally invasive surgical resection (VATS or RATS) in the thoracic surgery clinic for mediastinal mass between 2016 and 2022 were retrospectively evaluated. Patients who underwent sternotomy and thoracotomy for mediastinal mass resection were excluded.

Patients were analyzed in terms of gender, age, surgical method, mass location, operative time, operation side, complications, drainage amount, drainage time, pain score, length of hospital stay, and histopathological diagnosis parameters. Preoperative pulmonary function tests, biochemistry, hemogram, coagulation tests, and thyroid function tests (T3, T4, thyroid stimulating hormone were routinely evaluated in all patients. Mediastinal was examined using contrast-enhanced thoracic computed tomography (CT). alpha fetoprotein and ß-human chorionic gonadotropin levels were routinely measured in patients with anterior mediastinal masses. The histopathologic subtype evaluation of thymoma cases was performed according to the WHO classification (8).

Surgical Method

Mediastinal mass resection was performed by RATS (n=29) or VATS (n=23). All patients were intubated with a double-lumen endobronchial tube under general anesthesia. For anterior mediastinal masses, patients were positioned on the operating table in a 30° semilateral decubitus position (30° anterior inclination) with the side to be treated on the upper side. The right side was preferred for surgical intervention in anterior mediastinal masses. In the RATS procedure, the arms of the robot were draped after sterilization and draping of the patient. Port locations were adjusted. For the left arm port, an 8 mm incision was made at the intersection of the midclavicular line and the 5th intercostal space (ICA); for the video thoracoscope port, a 12 mm incision was made at the level of the midaxillary line 5th ICA, and for the right arm port, an 8 mm incision was made at the intersection of the anterior axillary line and the 3rd ICA. We used three ports in all our cases. The camera port was enlarged and removed when the specimen was removed.

CO_2 insufflation of 10 mmHg was used to expand the surgical field. The same procedure was performed in patients undergoing VATS for anterior mediastinal mass. Tumor and thymus tissue were removed en bloc in patients with preoperative diagnosis of thymoma or in whom thymoma could not be excluded, and in patients with a diagnosis of myasthenia gravis (MG). The mediastinal pleura was opened anterior to the phrenic nerve on the lower side. Dissection was performed upwards. The innominate vein was seen, and the thymus veins were seen after a careful dissection. It was divided by placing bilateral clips. Both thymic horns were freed and removed by pulling. All thymus tissue was removed by placing it in an endobag with the surrounding adipose tissue. A number 28 thoracic catheter was placed in the mediastinal region, and the incisions were closed according to the procedure. The lateral decubitus position was preferred for surgery in patients with a mass in the posterior or middle mediastinum. The RATS procedure was performed by placing trocars in the sixth ICA of the midaxillary line, at the intersection of the anterior axillary line and the fourth ICA, and at the intersection of the posterior axillary line and the eighth ICA. The fourth and seventh ICAs were used as the trocar sites for VATS.

Follow-up

All patients were admitted to the service on the first postoperative day. Intravenous non-steroidal anti-inflammatory drugs and paracetamol were routinely administered to all patients at four-hour intervals for postoperative pain control. Clavien-Dindo classification was used to classify surgical complications (9). Postoperative pain scores were calculated using the Numerical Rating Scale (NRS-11) (10). It was scored from 0 to 10, ranging from the least pain to the most severe pain. No pain was scored as 0, and unbearably severe pain was scored as 10. The NRS-11 score recorded 24 h postoperatively was used to measure postoperative pain (10). Thoracic drains were terminated when the total expansion was observed on the anterior-posterior chest radiograph (PA) of patients with daily drainage ≤100 mL/24 hours. Patients were routinely evaluated with hemograms, biochemical tests, and PA chest radiographs at 1 and 3 months postoperatively. Annual follow-up of the patients was performed using thoracic CT.

Statistical Analysis

Data were entered into the Statistical Package and analyzes were performed using commercial software (IBM SPSS Statistics, version 23.0. Armonk, NY: IBM Corp.). The normality of the distributions was determined by Shapiro Wilk's test. Normally distributed variables were calculated as mean, non-parametric variables not showing normal distribution were calculated as the median. The numerical variables were presented as the median and interquartile range (IQR). It was decided to use Student's t-test for comparisons of continuous variables between groups. The comparative analysis of qualitative variables was compared by the chi-square test. The Mann-Whitney U test was used to compare the demographic and clinic characteristics of VATS and RATS groups. A p-value <0.05 was considered significant.

Results

The mean age was 50.6±17.3 years (range: 17-80). 61.5% (n=32) of the patients were female and 38.5% (n=20) were male. 57.7% (n=30) of the mediastial masses were located in the anterior mediastinum. The distribution of clinical characteristics of patients undergoing VATS and RATS is summarized in Table 1.

The most common reason for admission was chest pain (46.2%, n=24). The most common postoperative pathology was thymoma (27%, n=14). According to the Masaoka staging system, [64.2% (n=9) stage 1, 21.4% (n=3) stage 2a], 14.2% (n=2) stage 2b thymomas were reported. Five of the patients with thymoma had a diagnosis of MG.

No surgical mortality was observed in any of our patients. Complications occurred in six patients (11.5%). Two (8.7%) of these complications occurred after VATS and four (13.8%) after RATS (p=0.682). When postoperative complications were evaluated according to the Clavien-Dindo classification, grade 1 complications (arrhythmia, atelectasis, prolonged air leak, wound infection) were seen in 4 patients and grade 2 (pneumonia, pulmonary embolism) in 2 patients. Negative suction with 5-10 mmHg pressure was applied in the patient with prolonged air leakage, and lung expansion was achieved. All patients improved with medical treatment.

Table 1. The distribution of clinical characteristics of patients undergoing VATS and RATS

		Total, n (%)	VATS, n (%)	RATS, n (%)	p
Gender	Male	20 (38.5)	9 (39.1)	11 (37.9)	1.000
	Female	32 (61.5)	14 (60.9)	18 (62.1)	
Location	Front	30 (57.7)	12 (52.2)	18 (62.1)	0.604
	Middle	6 (11.5)	2 (8.7)	4 (13.8)	
	Back	16 (30.8)	9 (39.1)	7 (24.1)	
Operating position	Lateral decubitus	33 (63.5)	16 (69.6)	17 (58.6)	0.600
	Semi-lateral decubitus	19 (36.5)	7 (30.4)	12 (41.4)	
Postoperative complications	No	46 (88.5)	21 (91.3)	25 (86.2)	0.682
	Yes	6 (11.5)	2 (8.7)	4 (13.8)	
Symptom	No	16 (30.8)	11 (47.8)	5 (17.2)	**0.038**
	Yes	36 (69.2)	12 (52.2)	24 (82.8)	
Conversion to thoracotomy	No	47 (90.4)	20 (87)	27 (93.1)	0.644
	Yes	5 (9.6)	3 (13)	2 (6.9)	

VATS: Video-assisted thoracoscopic surgery, RATS: Robot-assisted thoracoscopic surgery

Conversion to open surgery was required in a total of 5 patients [VATS group (n=3), 13% versus RATS group (n=2), 6.9%, p=0.644]. The distribution of complications, symptoms and surgical pathology results of VATS and RATS patients are summarized in Table 2.

The median length of hospital stay was five days [VATS; 4 days (IQR): 3-6] versus RATS; 5.5 days (IQR: 4-8), p=0.081]. The median drainage time was four days (IQR: 2-6), and the mean drainage amount was 110 mL (70 mL-190). There was no significant difference between the duration of the operation (for VATS; 75.7±18.4 min, for RATS; 73.5±18.0 min, p=0.674). Postoperative pain scores (NRS-11 score) were similar [median 2.19 (IQR: 1-3) for RATS and 2.20 (IQR: 1-3) for VATS, p=1.00]. A comparison of perioperative and postoperative variables in VATS and RATS is presented in Table 3.

The median drainage time was four days [VATS; 3 (2-5) versus RATS; 4.5 (3-7), p=0.133], and the mean drainage amount was 110 mL (70-190) (p=0.162). There was no significant difference between the duration of the operation (for VATS; 75.7±18.4 min, for RATS; 73.5±18.0 min, p=0.674). Postoperative pain scores were similar [median 2.19 (1-3) for RATS and 2.20 (1-3) for VATS, p=1.00].

The median follow-up time was 31 months. Adjuvant postoperative radiotherapy was performed in four patients due to capsular invasion and in one patient due to stage 2 type B3. In the 3rd year of follow-up, recurrence was observed in one patient (1.9%) in the RATS group. Reoperation was performed by the transsternal approach.

Discussion

RATS and VATS, which are minimally invasive surgical methods, have recently become increasingly preferred in the surgical treatment of mediastinal masses because of their advantages. Compared to traditional open surgical methods, the generally accepted advantages include reduced operation time, less postoperative pain, rapid postoperative recovery, lower complication rate, lower risk of infection, and a better cosmetic appearance (11-13).

The introduction of VATS in the diagnosis of pleural and parenchymal diseases of the lung marked the beginning of a new era in the use of minimally invasive techniques in thoracic surgery. The adoption of VATS as the first choice for cancerous resections of the lung, thymectomy, and mediastinal tumor resections, which require more complex surgical procedures, has greatly increased our experience in minimally invasive methods (7). On the other hand, RATS provides a safer dissection thanks to its high image quality and 360-degree rotating articulated endo-wristed instruments. This technique especially provides great convenience to the surgeon in dissection of locally invaded mediastinal tumors. A limited number of studies have compared the outcomes of VATS and RATS in surgical treatment of mediastinal masses. Studies comparing RATS and VATS procedures were mostly based on thymectomy outcomes. In these studies, RATS was reported to be superior to VATS with less complication rate, less hospitalization time, and less drainage amount (12,14,15). Recurrence rates after thymectomy are reported to be 3-9% in the literature (16). In our series, one patient (1.9%) in the RATS group had local recurrence at 33 months postoperatively. The patient was reoperated by sternotomy.

Early complication rates in mediastinal mass surgery have been reported to be between 5%-14% for VATS and 3%-13% for RATS (6,7). In studies comparing VATS and RATS procedures for the resection of mediastinal masses, the overall postoperative complication rates after RATS have been reported to be significantly lower (7,17,18). In our study, unlike the literature, the postoperative complication rates of both procedures were similar (8.7% vs 13.8%, p=0.682).

Zeng et al. (7) reported that unplanned thoracotomy rates were significantly higher in the VATS group (p=0.04). In the study mentioned above, the total duration of hospitalization was significantly lower in the RATS group. In a study analyzing the early results and efficacy of RATS and VATS regardless of histology, the unplanned thoracotomy rate was 15% for VATS and 5% for RATS. Mortality was 2.3% for VATS and 1.0% for RATS. In that study, RATS was reported to have better outcomes compared with VATS, with a lower incidence of unplanned thoracotomy and a shorter postoperative hospital stay (3.8 d vs. 4.3 d, p=0.01) (19). Another study reported that RATS provided a shorter duration of hospitalization and less amount of postoperative pleural drainage compared with the VATS

Table 2. The distribution of complications, symptoms, and surgical pathology results of patients undergoing VATS and RATS

		Total, n (%)	VATS, n (%)	RATS, n (%)
Pathology	Bronchogenic cyst	1 (1.9)	1 (4.3)	0 (0)
	Bronchogenic cyst	1 (1.9)	1 (4.3)	0 (0)
	Epithelioid hemangioendothelioma	1 (1.9)	0 (0)	1 (3.4)
	Ganglioneuroma	1 (1.9)	0 (0)	1 (3.4)
	Cavernous hemangioma	1 (1.9)	0 (0)	1 (3.4)
	Lipoma	1 (1.9)	1 (4.3)	0 (0)
	Mesothelial cyst	1 (1.9)	1 (4.3)	0 (0)
	Müllerian cyst	1 (1.9)	1 (4.3)	0 (0)
	Teratoma	4 (7.7)	2 (8.7)	2 (6.9)
	Paraesophageal cyst	2 (3.8)	1 (4.3)	1 (3.4)
	Pericardial cyst	12 (23.1)	5 (21.7)	7 (24.1)
	Schwannoma	6 (11.5)	3 (13)	3 (10.3)
	Solitary fibrous tumor	1 (1.9)	0 (0)	1 (3.4)
	Thymic hyperplasia	5 (9.6)	2 (8.7)	3 (10.3)
	Thymoma micronodular	2 (3.8)	1 (4.3)	1 (3.4)
	Thymoma type A	3 (5.7)	3 (13)	0 (0)
	Thymoma type AB	5 (9.6)	1 (4.3)	4 (13.8)
	Thymoma type B1	2 (3.8)	1 (4.3)	1 (3.4)
	Thymoma type B3	2 (3.8)	0 (0)	2 (6.9)
Postoperative complications	No	46 (88.5)	21 (91.3)	25 (86.2)
	Arrhythmia	1 (1.9)	0 (0)	1 (3.4)
	Atelectasis	1 (1.9)	1 (4.3)	0 (0)
	Pneumonia	1 (1.9)	0 (0)	1 (3.4)
	Pulmonary embolism	1 (1.9)	0 (0)	1 (3.4)
	Prolonged air leakage	1 (1.9)	1 (4.3)	0 (0)
	Wound site infection	1 (1.9)	0 (0)	1 (3.4)
Presenting symptom	No symptoms	16 (30.8)	11 (47.8)	5 (17.2)
	Chest pain	24 (46.2)	8 (34.8)	16 (55.2)
	Shortness of breath	5 (9.6)	0 (0)	5 (17.2)
	Cough	3 (5.8)	2 (8.7)	1 (3.4)
	Back pain	3 (5.8)	1 (4.3)	2 (6.9)
	Difficulty swallowing	1 (1.9)	1 (4.3)	0 (0)

VATS: Video-assisted thoracoscopic surgery, RATS: Robot-assisted thoracoscopic surgery

Table 3. The comparison of perioperative and postoperative variables in VATS and RATS groups

	Total		VATS		RATS		p
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	
Age	52	50.6±17.3	23	50.3±17.9	29	50.9±17.2	0.913
Tumor diameter (mm)	52	56.0±40.4	23	54.9±29.8	29	57.1± 37.0	0.919*
Operation duration (minute)	52	74.5±18.0	23	75.7±18.4	29	73.5±18.0	0.674
The length of stay hospital	52	5 (3-7)	23	4 (3-6)	29	5.5 (4-8)	0.081*
Chest tube removal (day)	52	4 (2-6)	23	3 (2-5)	29	4.5 (3-7)	0.133*
Chest tube drainage (mL)	52	110 (70-190)	23	90 (60-140)	29	130 (80-205)	0.162*

*: According to Mann-Whitney U test [descriptive statistics were shown as median (IQR)], IQR: Interquartile range, VATS: Video-assisted thoracoscopic surgery, RATS: Robot-assisted thoracoscopic surgery, SD: Standard deviation

approach. In the same study, only one patient in the VATS group needed conversion to open surgery (20).

There was no surgical mortality in our series. There was no significant difference between the duration of the operation (VATS; 75 min vs RATS; 73 min). The length of the hospital stay was five days (VATS; 4 days vs RATS; 5.5 days, p=0.08). Conversion to open surgery was required in 5 patients. When we compared the RATS and VATS procedures, unlike the literature, we did not observe any difference in terms of the amount of bleeding, duration of operation, duration of hospitalization, amount of postoperative pleural drainage, and conversion rate to open surgery. Given that our RATS procedure results align with the literature, this could be attributed to our center has extensive experience in VATS applications. Major vascular bleeding is the most feared intraoperative complication in both VATS and RATS procedures. The RATS procedure requires more safety precautions than VATS because transitioning from RATS to open thoracotomy in emergencies takes longer than VATS (1). None of our patients had a major vascular injury or blood loss requiring transfusion.

Study Limitations

The examination of a heterogeneous group with a retrospective design was the main limitation of our study. Since the number of patients was not sufficient, we could not compare the same type of tumor in the same location. However, the strengths of the study are that the same physicians performed standard surgical procedures, and the number of patients was sufficient for an accurate evaluation.

Conclusion

The minimally invasive surgical methods RATS and VATS are effective and safe procedures offering many advantages in treating mediastinal masses. RATS and VATS procedures have similar results regarding complication rate, length of hospital stay, and duration of surgery.

Ethics Committee Approval: The ethics committee approval of this study was obtained from the Ethics Committee of Sakarya University Faculty of Medicine Non-Interventional Ethics Committee and was conducted following the principles of the Declaration of Helsinki (approval number: E-71522473-050.01.04-216185-09, date: 31.01.2023).

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Status of Appendiceal Neoplasms in Acute Appendicitis Cases

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ABSTRACT

Introduction: Appendiceal neoplasms (AN) are exceedingly rare and mostly diagnosed incidentally during appendectomy due to non-specific clinical manifestations. Our study focused on assessing the clinical and postoperative histopathological features of ANs to differentiate them from acute appendicitis (AA) diagnosis in order to prevent complications and metastasis in aggressive malignancies.

Methods: In this retrospective study, we analyzed 2,906 patients who underwent a appendectomy. We compared the demographic characteristics, imaging, and preoperative laboratory findings and postoperative histopathology results between the groups with AN and AA.

Results: The prevalence of AN was found to be 2.82% (n=82). We observed a significant difference in age between patients diagnosed with AN and those with AA, with patients being notably older. The rate of perforation and diverticula was also increased in patients with neoplasms. Low-grade mucinous adenoma (39.02%) was the most common neoplasm, followed by precancerous serrated adenoma (28.04%) and carcinoid tumor (21.95%), respectively. Moreover, the mean diameter of carcinoid tumors was 6.32±4.69 mm and 2 patients with >20 mm lesion diameter underwent right hemicolectomy. Carcinoid tumors were mostly located at the tip of the appendix. In addition, no lymphovascular invasion or distant metastasis was observed in any of the patients.

Conclusion: Primary ANs are exceedingly rare and easily overlooked, the increased incidence of major complications such as perforation should be taken into consideration with AA-like clinical presentation in AN patients. Thus, preoperative laboratory and especially radiological outcomes should be carefully evaluated.

Keywords: Appendiceal neoplasms, acute appendicitis, carcinoid tumor, low grade mucinous neoplasm

Introduction

Appendiceal neoplasms (AN) represent about 0.9 to 1.4% of all AN (1). Due to unevaluated postoperative lesions, the rate of actual appendix mass is estimated to be up to 5% (2). AN represent a broad heterogeneous group classified as epithelial and non-epithelial (3). Mucinous neoplasms that are histologically classified as low or high grade are the most common (85%) malignancy of all epithelial ANs, while neuroendocrine tumors comprise the majority of non-epithelial tumors with increased incidence recently (4,5).

The clinical presentation of ANs is usually non-specific with typical or atypical acute appendicitis (AA)-like symptoms even hormonally active carcinoid neoplasms (3). Thus, the absence of pathognomonic findings mostly results in delayed diagnosis or incidental diagnosis during appendicectomy (5). ANs are rarely aggressive tumors and their prognosis depends on stage, histological type. Although nodal involvement and distant metastases are less frequently documented during diagnosis, appendiceal adenocarcinoma is significantly associated with lower 5-year survival rates (1). Therefore, appropriate surgical resection is still the standard suggested curative option for ANs without distant metastasis (6). However, in some instances, further surgery such as right hemicolectomy may be required, particularly in neuroendocrine neoplasms with potentially malignant larger lesions (7).

Although the majority of ANs are associated with better overall prognosis, various tumors exhibit higher perforation risks, malignant potential with possible regional and distant metastases. Accurate and prompt diagnosis and effective management of surgical approach are crucial in patients with ANs (1,5). Therefore, our objective was to assess the clinical and postoperative histopathological features of ANs that differ from the diagnosis of AA to prevent complications associated with aggressive malignancies.

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Methods

Conducted at the Department of Surgery in University of Health Sciences Turkey, İstanbul Training and Research Hospital, this study took place from January 2016 to December 2021. The study protocol received approval from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 2847, date: 04.06.2021), and informed consent was obtained from all participating patients. A total of 2,906 patients who underwent appendectomy were retrospectively evaluated in this study. We compared the demographic characteristics, imaging, and preoperative laboratory findings of the patients as well as the postoperative histopathology results (both from laparoscopic and open appendectomy procedures) between the groups with AN and AA.

Patients in this study exhibited symptoms such as appetite loss, nausea, and vomiting. The surgeon assessed the clinical and physical manifestations of AA, with at least one clinical finding indicating a likelihood of AA. These clinical findings included right lower abdominal pain, percussion, and rebound tenderness, and localized and diffuse rigidity of the abdominal wall. A definitive diagnosis was established through histopathological evaluation.

Venous blood samples from the patients were collected for cell blood count analysis. The hematological parameters were assessed using a hematology analyser (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA). Additionally, serum samples were obtained for biochemical analysis which was conducted through electro-chemiluminescence immunoassay on the Beckman Coulter Unicel DXI 800 analyzer.

Statistical Analysis

The data analysis was conducted using SPSS software for Windows (v21.0; IBM, Armonk, NY, USA). Descriptive statistics, including mean, standard deviations, medians, interquartile range, frequency distributions, and percentages, were employed to summarize both individual and aggregate data. The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. For variables that did not follow a normal distribution, the Mann-Whitney and Kruskal-Wallis tests were employed to compare between groups. The evaluation of categorical variables was performed using the chi-square test. P-values below 0.05 were considered statistically significant.

Results

Out of the 2,906 patients included in this study, 1830 (63.00%) were male and 1076 (37.00%) were female, resulting in a male-to-female ratio of 1.70. The median age of all patients was 32.00 years. In our study sample, the overall prevalence of AN was 2.82% (n=82). There was no significant difference observed between genders within the groups. However, patients with AN were notably older than those diagnosed with AA. Additionally, AA patients had significantly higher rates of perforation and diverticulosis, as shown in Table 1.

Upon analyzing the laboratory findings, it was observed that the white blood cell count and neutrophil values were significantly higher in the AA group (p-values: 0.001, 0.005, respectively). Furthermore, patients with AN had significantly elevated mean platelet volume (MPV) compared with the AA group (p-value: 0.001) (Table 2).

The overall incidence of perforated appendicitis in the entire patient cohort was determined to be 6.4% (n=188). Specifically, within the AA group, the incidence of perforated appendicitis was found to be 6.3% (n=178). However, among patients with AN, the perforation rate increased to 13.8% (n=10), indicating a statistically significant difference (p-value: 0.032). Furthermore, the prevalence of appendiceal

Table 1. Comparison of age and gender between groups

		Appendicitis (n=2,824)	Neoplasia (n=82)	p-value
Age (median - IQR)		32.00-15.00	43.00-31.00	0.001
Gender	Female (n, %)	1,040 (36.8%)	36 (43.9%)	0.191
	Male (n, %)	1,784 (63.2%)	46 (56.1%)	
Diverticula	No (n, %)	2,763 (97.8%)	73 (89%)	0.001
	Yes (n, %)	61 (2.2%)	9 (11%)	
Perforation	No (n, %)	2,646 (93.6%)	72 (84.1%)	0.032
	Yes (n, %)	178 (6.3%)	10 (13.8%)	

IQR: Interquartile range

Table 2. Comparison of laboratory outcomes between the AA and groups

Laboratory results	Appendicitis (n=2,824)	Neoplasia (n=82)	p-value
WBC ($x10^9$/L), (median - IQR)	14.00-5.45	12.00-6.00	0.001
Neutrophil ($x10^9$/L), (median - IQR)	10.98-5.45	9.20-5.37	0.005
Lymphocyte ($x10^9$/L), (median - IQR)	1.80-1.18	1.80-1.20	0.105
Neutrophil/lymphocyte, (median - IQR)	5.90-6.03	5.28-5.56	0.462
Platelet ($x10^9$/L), (median - IQR)	247.00-84.00	248.00-87.00	0.754
MPV (fL), (median - IQR)	8.30-1.50	8.90-1.70	0.001
Bilirubin (mg/dL) (median - IQR)	0.60-0.20	0.65-0.30	0.174

AA: Acute appendicitis, IQR: Interquartile range

Table 3. The distribution of neoplasms

Neoplasia types	According to the number of total samples	According to the number of neoplasia samples
Mesothelial cysts (n, %)	1 (0.03%)	1 (1.21%)
Low-grade mucinous neoplasm (n, %)	32 (1.10%)	32 (39.02%)
Hyperplastic polio (n, %)	3 (0.09%)	3 (3.63%)
Intramucosal carcinoma (n, %)	1 (0.03%)	1 (1.21%)
Carcinoid tumor (n, %)	18 (0.61%)	18 (21.95%)
Mucosal (n, %)	2 (0.06%)	2 (2.42%)
Over adenocarcinoma inflitration (n, %)	1 (0.03%)	1 (1.21%)
Mucinous adenocarcinoma (n, %)	1 (0.03%)	1 (1.21%)
Sessile serrated adenoma (n, %)	23 (0.79%)	23 (28.04%)

diverticulosis (AD) was found to be significantly higher in AN patients compared with other groups (p-value: 0.001).

In the current study, the overall prevalence of neoplasm-associated lesions was found to be 2.82% (n=82). Among these lesions, low-grade mucinous neoplasms had the highest frequency, accounting for 39.02% of cases. Precancerous serrated adenoma followed closely with a rate of 28.04%, while carcinoid tumors were identified in 21.95% of cases (Table 3).

The mean tumor diameter was 6.32±4.69 mm (range: 1.0-18.0 mm) in patients who underwent laparoscopic or open appendectomy and were diagnosed incidentally with carcinoid tumors. After histopathological assessment, 2 patients with >20 mm lesion diameter underwent right hemicolectomy. Tumors were located at the tip of the appendix in 15 (83.3%) patients, at the body of the appendix in 2 (11.1%) patients, and the remaining 1 (5.5%) were located in the base of the appendix. The surgical margin was negative in all cases. Ki-67 proliferation was found to be high (≥3%) in 11.1% of cases (n=2), and low (<3%) in 88.9% of cases (n=16) (Table 4). Immunohistochemical evaluation revealed synaptophysin and chromogranin-A positivity in all patients. While perineural invasion was detected in 3 patients (16.6%), no lymphovascular invasion or distant metastasis was observed in any of the patients. A total of 5 patients (27.7%) had subserosa invasion, 6 patients (33.3%) had mesoappendiceal invasion, 4 patients (22.2%) had local invasion into the muscular layer, 1 patient (5.5%) had submucosal invasion, and 1 patient (5.5%) had mucosal invasion.

Table 4. Clinical characteristics of patients with carcinoid tumors

Parameter		Carcinoid tumors (n=18)
Age (mean ± SD)		35.11±14.19
Gender	Female	8 (44.44%)
	Male	10 (55.56%)
Localization	Tip	15 (83.33%)
	Body	2 (11.11%)
	Base	1 (5.55%)
Ki -67 (mean ± SD)	<3%	16 (88.88%)
	>3%	2 (11.12%)
Tumor diameter (mean ± SD)		6.32±4.69

SD: Standard deviation

Discussion

Primary ANs tend to occur more commonly in middle-aged or older patients with the exception of neuroendocrine tumors. Neuroendocrine tumors relatively occur in younger ages than other ANs (1). Tan et al. (6) reported a mean age of 53 years and of the patients 45% were male, 55% were female in their retrospective study with participation of 685 AN patients. Similarly, Kunduz et al. (8) reported significantly greater age (33.24 years vs 44.5 years) in AN patients (n=28) compared to AA patients among 3,554 appendectomies between 2011 and 2017 years. On the other hand, Lamberti et al. (9) documented a median age of 29 years in 339 patients diagnosed with appendiceal neuroendocrine tumors. Consistent with our study findings, it was observed that patients with AN were significantly older than those with AA. Additionally, among AN patients, individuals diagnosed with appendiceal neuroendocrine tumors had an average age of 35.11 years.

Because AA is an inflammatory disease, the severity of AA is associated with increased leukocyte and neutrophil counts as inflammatory markers (10). Furthermore, inflammation can contribute to an upsurge in platelet production, which in turn may lead to variations in platelet volume. As a result, high-grade inflammation characterized by excessive consumption can potentially lead to decreased MPV levels (11). Researchers observed an elevated risk for developing AA in patients with increased leukocyte (12). Likewise, Xharra et al. (13) observed elevated leukocyte and neutrophil counts in AA patients. Additionally, Ceylan et al. (14) reported lower MPV levels in 363 AA patients compared with healthy controls. Consistent with these findings, our study found that the mean leukocyte, neutrophil, and lymphocyte counts in the AN group were statistically lower than those in the AA group. Furthermore, the MPV values measured in the group were statistically higher than those in the AA group.

The neoplastic tumor cells or mucin production may cause lining and obstruction of the lumen, moreover, lining may lead to herniation into the muscularis propria, and perforation occurs (3). Furthermore, perforation may result with pseudomyxoma peritonei, particularly in mucinous neoplasms (15). Kunduz et al. (8) documented perforation and plastron appendicitis rates of 25% (n=7) and 3.5% (n=1), respectively, in 28 AN patients. Similarly, Tajima et al. (2) reported a perforation rate of 17.6% (n=3) among 17 patients diagnosed with AN. Additionally, Honoré et al. (15) found higher rates of perforated appendicitis (75%) in 25 patients

with mucinous neoplasms, providing further support. Furthermore, there are limited published data regarding the association between AD and neoplastic processes. In Kallenbach et al. (16), a significant association between AN and 43.6% of 39 AD cases were reported among 4,413 appendectomies. Additionally, Marcacuzco et al. (17) identified a neoplastic association in 7.1% of 42 patients diagnosed with AD out of a total of 7,044 appendectomies. In our study, a statistically higher rate of perforation was observed in the group. Furthermore, the incidence of neoplasms associated with AD was found to be 11.0% among the 2906 appendectomies conducted in this study.

The published data indicate that the prevalence of AN diagnosed during surgery ranges from 0.7% to 5% (2). In a retrospective study conducted by Hosseinzadeh et al. (18) involving 4,800 patients between 2010 and 2014, the prevalence of AN was reported as 1.8% (n=86). The researchers observed that carcinoid tumors were the most frequently encountered type of AN in their study (18). Similarly, Tajima et al. (2), in a study involving 803 appendectomy cases, documented an incidence of 2.3% (n=17) for AN. Among these cases, intramucosal neoplasms were found to be the most common type of AN (2). On the other hand, while mucinous carcinoma was the most frequent neoplasm in a study conducted by Tan et al. (6), in another study Kunduz et al. (8) reported neuroendocrine tumors as the most common type of ANs. Moreover, Lietzén et al. (19) reported an overall AN prevalence of 1.24% among 472 AA patients, and researchers highlighted a significantly increased tumor risk in complicated AA. The present study aligns with the reported data, indicating an overall prevalence of 2.8% for AN. Additionally, according to the published data, mucinous neoplasms and carcinoid tumors are the most commonly identified AN. In our study, low-grade mucinous neoplasm was the most frequently determined malignancy, followed by sessile serrated adenoma.

Appendiceal carcinoid tumors are considered a rare etiology of AN, with reported incidences ranging from 0.3% to 0.9% among appendectomy cases. Carcinoid tumors generally emerge in young patients, and the majority of lesions are typically localized at the tip of the appendix (1). Furthermore, it has been observed that an increased tumor size (>2 cm) in carcinoid tumors is linked to a heightened risk of metastasis (18). In't Hof et al. (20) reported a mean age of 32.7 years in patients with carcinoid tumors, and researchers also noted a carcinoid tumor prevalence of 0.47% after 1,485 appendectomies. In addition, a patient with a tumor larger than 2 cm underwent right hemicolectomy (20). It has also been recommended in published data to perform right hemicolectomy in malignant carcinoids lesions larger than 2 cm in diameter (21). Tchana-Sato et al. (22) documented a mean age of 29.2 years in 5 patients with carcinoid tumors after 1,237 appendectomies and lesions were all localized at the tip of appendix. In another study consisting of 50 patients diagnosed with carcinoid tumors between 1994 and 2010 Murray et al. (23) reported a median tumor diameter of 5 mm with all negative margins. Most (76%) of the tumors were determined to be localized at the tip of the appendix in the same study. A researcher also stated that 2 patients underwent right hemicolectomy, and no regional lymph node or distant metastasis was documented (23). Consistent with the data, this study found that patients with carcinoid tumors had a mean tumor diameter of 6.32±4.69 mm, and all cases exhibited negative margins. The lesions were mostly (83.3%) localized at the tip of the appendix. Two patients with >20 mm lesion diameter underwent right hemicolectomy. Additionally, no lymphovascular invasion or distant metastasis was observed in any of our patients.

Study Limitations

Due to the small number of tumoral lesions, rare pathologies were either unobserved or rarely.

Conclusion

Although primary ANs are exceedingly rare, it should be taken into consideration that tumoral obstruction in the appendiceal lumen may present with AA-like clinical presentation. As demonstrated in the present study, due to the increased incidence of major complications such as perforation in patients with ANs, laboratory and radiological findings should be attentively evaluated. Additionally, incidentally detected macroscopic AD, which is also rare and easily overlooked, should be referred to appendectomy due to the increased association of malignant potential. However, appendectomy specimens preperatively manifest normal macroscopic features, adequate histopathological examination is vital for an accurate and early diagnosis of possible neoplasms and appropriate treatment approaches.

Ethics Committee Approval: The study protocol received approval from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 2847, date: 04.06.2021).

Informed Consent: Informed consent was obtained from all participating patients.

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Authorship Contributions: Surgical and Medical Practices - E.Ç., M.M.S., Ş.Ç., M.D., E.Y., U.O.İ.; Concept - E.Ç.; Design - E.Ç., U.O.İ.; Data Collection or Processing - E.Ç., M.M.S., Ş.Ç., M.D., E.Y., U.O.İ.; Analysis or Interpretation - E.Ç., M.M.S., Ş.Ç., M.D., E.Y., U.O.İ.; Literature Search - E.Ç., M.M.S., Ş.Ç.; Writing - E.Ç., M.M.S., Ş.Ç., M.D., E.Y., U.O.İ.

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Evaluation of Salivary Glands by Ultrasonography and Inflammatory Markers in Children with Autoimmune Thyroiditis

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ABSTRACT

Introduction: Although more common in adults, autoimmune thyroiditis (AT) is one of the most common thyroid diseases in children and adolescents. Salivary gland involvement has been described in many studies of patients with AT. Several inflammatory scores are used to assess the inflammatory status of patients with systemic autoimmune diseases. We aimed to sonographically evaluate the parotid and submandibular salivary glands with inflammatory parameters in patients with AT in our study.

Methods: Our study population consisted of 37 consecutive pediatric AT patients and 29 healthy control subjects. Ultrasonographic and laboratory evaluations of the study population were performed. Jamovi and MedCalc software were used to analyze the data.

Results: The volume of the thyroid gland in the patients was significantly higher than that in the control group (p=0.030), while there was no difference in the volume of the salivary glands. Multiple logistic regression analysis was planned to assess the predictability of salivary gland involvement in patients with the disease. Both systemic immune-inflammation index (SII) and pan-immune inflammation value (PIV) were found to be predictors of salivary gland involvement in AT patients.

Conclusion: We found that both SII and PIV inflammatory markers are predictive of salivary gland parenchymal changes in patients with AT, and SII is likely to be more valuable than PIV at this time.

Keywords: Autoimmune thyroiditis, gland, parotid, submandibular, SII, PIV

Introduction

Autoimmune thyroiditis (AT) is the most common disease of the thyroid gland in the pediatric age group. Although a combination of genetic, environmental, and immune factors are thought to play a role, the exact cause of AT is not fully understood. While AT can occur at any age, including pediatric age, the mechanisms underlying its development in children are similar to those in adults. AT can occur on its own or can be associated with other autoimmune diseases. The combination of AT with specific autoimmune disorders is known as autoimmune polyglandular syndromes (1,2). However, in some cases, there can be associated to the involvement of the salivary glands. The involvement of the salivary glands in AT is relatively rare but has been the subject of reports in the literature (3-5). However, as far as we know, salivary glands in patients with AT have not been sonographically evaluated in the literature.

Ultrasound (US) is an excellent choice for the initial evaluation of the salivary gland in pediatric patients and is an easily accessible and non-invasive method for evaluating superficial structures with good resolution. It is the first choice for pediatric patients as it does not contain the radiation. Normal submandibular and parotitis glands have homogeneous parenchyma on US and are hyperechoic compared to adjacent muscles, and the degree of echogenicity may vary in proportion to the amount of glandular adipose tissue (6).

In recent years, several leukocyte-based inflammatory markers have been identified that can provide valuable insight into an individual's inflammatory status, including the systemic immune-inflammation index (SII), monocyte/high-density lipoprotein ratio, platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio, neutrophil/lymphocyte ratio (NLR) and pan-immune value (PIV). There is evidence that these inflammatory markers may also be useful in assessing the inflammatory status of patients with systemic autoimmune diseases (7-9).

No US-based study has been conducted on parotid and submandibular gland involvement in AT disease in pediatric patients. The main objective of this study was to evaluate parotid and submandibular gland parenchyma in patients with AT using US and inflammatory parameters.

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Methods

The study participants consisted of patients followed up with a diagnosis of AT in the pediatric endocrinology clinic and a control group. Consecutive patients with AT who were under 18 years of age and had parental consent were eligible for inclusion in the study. AT was diagnosed using radioimmunoassay results for anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (ATG), and thyroid-stimulating hormone (TSH). The control group consisted of participants who were examined with neck US for any reason and whose thyroid function tests (TFT) and TSH values were normal for the last 1 month. Patients who had another systemic disease and were receiving drug therapy were excluded from the study. The study was conducted in accordance with the Declaration of Helsinki and the guidelines for good clinical practice. The study was approved by the İnönü University Scientific Research and Publication Ethics Committee (approval number: 2020/491, date: 17.03.2020).

Ultrasonographic Evaluation

US was examined with a Logiq s8 (GE healthcare, USA) device using a linear probe with a frequency of 14 MHz. US was performed on all participants by the same pediatric radiologist unaware of their clinical and biochemical status. In the US examination, parenchyma echo structures and dimensions of the thyroid gland, submandibular gland, and parotid gland were evaluated with gray scale. Vascularity of the thyroid gland, submandibular gland, and parotid gland was evaluated subjectively with Doppler US. The thyroid gland was evaluated in the supine position and the neck in extension. The gain settings of the US scanner were adjusted so that the lumens of the carotid artery and internal jugular vein were echo-free. Normal thyroid parenchyma was defined as homogeneous and relative hyperechogenicity compared with adjacent muscle tissue. On gray scale US, abnormal parenchymal features of the thyroid gland were evaluated as heterogeneous echo and/or hypoechoic areas.

For each thyroid lobe, the mediolateral length (MLL) and the anteroposterior length (APL) were measured in the axial section and the inferior superior length (ISL) in the sagittal axis. The volume of each lobe was estimated using a standard geometric formula of APL x MLL x ISL x 0.523. The volume of the whole thyroid gland was calculated as the sum of the two lobes.

The submandibular glands were evaluated by US with the patient's head slightly raised and the parotid gland in the supine position with the patient's head facing the contralateral side. Each salivary gland size was evaluated for its echogenicity in at least two perpendicular planes. For each gland, MLL and APL were measured in the axial section and ISL in the sagittal axis. The standard geometric formula APL x MLL x ISL x 0.523 was used to estimate the volume of each gland. Gland volume was calculated as the average of both gland volumes. In the gray scale US, coarse echoes and hypoechoic areas in the gland parenchyma were considered abnormal parenchymal features (Figure 1).

Laboratory Evaluation

The results of complete blood count and TFT were obtained from the files of patients who were examined in the pediatric endocrine clinic. Two biomarkers of inflammation were calculated using the following formulae:

SII = platelet count x neutrophil count/lymphocyte count (10),

PIV = platelet count x neutrophil count x monocyte count/lymphocyte count (11).

Statistical Analysis

Jamovi (version 2.3.28) and MedCalc (version 20.027) software were used to analyze the data. The Kolmogorov-Smirnov test was used for the data distribution. Student's t-test or Mann-Whitney U test was used for continuous variables and chi-square test for categorical variables, depending on the distribution of the data. Multiple logistic regression analysis was used to assess the salivary gland involvement. Age, sex, SII, and PIV parameters were included as univariate parameters in the regression analysis. Since SII and PIV parameters are obtained from similar variables, two modeling (for PIV and SII) were performed to avoid multicollinearity. Pairwise receiver operating characteristic (ROC) analysis was used to compare the two models [area under the curve (AUC), Youden index]. P-value <0.05 was considered statistically significant.

Results

The demographics of the study population are shown in Table 1. There were no statistical differences between the groups for age, sex, and laboratory parameters (p>0.05, for all).

The ultrasonographic findings of the thyroid and salivary glands of the patients and the control group are given in Table 2. While the volume of the thyroid gland was significantly higher in the patients than in the control group (p=0.030), there was no difference in the volume of the salivary glands. However, salivary gland parenchyma involvement and vascularity in the patient group were significantly different from those in the control group.

Multiple logistic regression analysis was used to evaluate the predictability of salivary gland involvement in patients with the disease. To avoid multicollinearity in the analysis, two models were performed (for model 1: SII, model: 2 for PIV). In the analysis, SII [odds ratio (OR): 1.002, p=0.046] value was found to be significant in predicting salivary gland involvement in model I, and PIV (OR: 1.002, p=0.040) value was also found to be an independent predictor of salivary gland involvement

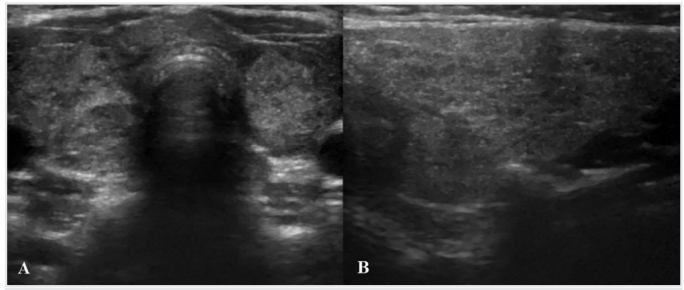

Figure 1. Heterogeneous nodular appearance of the thyroid parenchyma in a 9-year-old girl with autoimmune thyroiditis (A). Heterogeneous parenchyma accompanied by hypoechoic areas in the left parotid gland (B)

Table 1. Demographics and laboratory parameters of the study population

	The control group, (n=29)	Patients group, (n=37)	p-value
Age, months	14.2±2.1	14.6±2.3	0.489
Gender, female, (n, %)	17 (58.6%)	28 (75.7%)	0.140
WBC, 10^3/uL	9.4±5.7	7.6±2.3	0.087
Hemoglobin, g/dL	13.5±1.2	13.2±1.1	0.424
Platelet, 10^3/uL	334.7±69.0	312.0±61.1	0.162
Lymphocyte, 10^3/uL	2.7±0.7	2.4±0.7	0.060
Monocyte, 10^3/uL	0.7±0.3	0.6±0.2	0.244
Neutrophile, 10^3/uL	5.8±5.6	4.4±2.2	0.181
SII	608.8±455.7	587.6±510.2	0.861
PIV	406.1±402.9	406.0±672.8	0.999
TSH, mIU/L	-	6.0±10.0	-
fT4, ng/dL	-	3.9±16.6	-
Anti-TPO, IU/mL	-	835.3±430.7	-
ATG, IU/mL	-	622.4±857.7	-

ATG: Anti-thyroglobulin, TSH: Thyroid-stimulating hormone, fT4: Free thyroxine, WBC: White blood count, SII: Systemic immune-inflammation index, PIV: Pan-immune-inflammation value, TPO: Thyroid peroxidase

Table 2. Ultrasonographic measurements of the study population

	The control group, (n=29)	Patients group, (n=37)	p-value
Thyroid volume, mm^3	5.3±4.5	7.9±4.5	0.030
Parotid volume, mm^3	10.3±4.6	10.9±5.3	0.636
Submandibular volume, mm^3	8.6±4.6	9.0±4.1	0.705
Thyroid parenchyma	0 (0%)	30 (81.1%)	<0.001
Thyroid vascularity	0 (0%)	19 (51.4%)	<0.001
Parotid parenchyma	3 (10.3%)	16 (43.2%)	0.003
Parotid vascularity	2 (6.9%)	10 (27.0%)	0.036
Submandibular parenchyma	3 (10.3%)	20 (54.1%)	<0.001
Submandibular vascularity	4 (13.8%)	10 (27.0%)	0.099

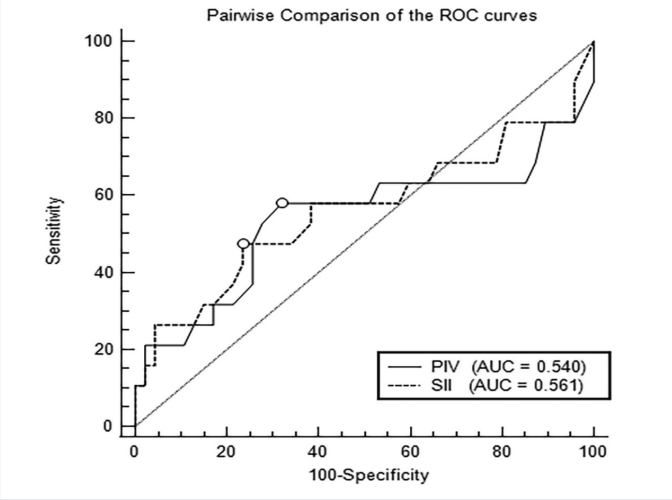

Figure 2. Pairwise comparison of the PIV and SII values in the prediction of salivary gland involvement in patients with thyroiditis (z-statistics: 0.143, differences between AUCs: 0.021, p=0.887)

SII: Systemic immune-inflammation index, PIV: Pan-immune-inflammation value, AUC: Area under the curve, ROC: Receiver operating characteristic

in model 2. Pairwise ROC analysis was performed to compare both parameters in predicting the salivary gland involvement. In the analysis, although SII gave a higher AUC value, there was no statistical difference in terms of both parameters (Figure 2).

Discussion

The main findings of our study were: (i) salivary gland parenchymal changes were significantly higher in patients with AT than in the control group, but there was no difference in salivary gland volumes; (ii) salivary gland involvement could be predicted by SII and PIV parameters in the regression analysis for salivary gland involvement; (iii) when comparing both parameters, there was no statistical difference in the pairwise ROC analysis. The disease, which is more common in women even before puberty, can result from defects in immune regulation or lymphocyte infiltration of the thyroid. In the majority of patients, antibodies (abs) can be detected against various thyroid-specific antigens. Whether antibody-mediated immune mechanisms contribute to the onset, progression, or pathogenesis of AT remains unclear. Anti-TPO and ATG are diagnostic markers of the underlying autoimmune destruction of the thyroid gland and are also found in the majority of patients (12).

The thyroid gland is histologically similar to the lacrimal and salivary glands. Studies of salivary gland involvement in people with AT have suggested that common mechanisms may be at work in the development of thyroiditis and salivary gland immune disease (5,13-15). It has been shown that an immunological imbalance in the salivary glands leads to secretory dysfunction not only in Sjögren's syndrome (SS) but also in other autoimmune diseases such as psoriasis (16), rheumatoid arthritis (17) and systemic sclerosis (18). Genetic and immunopathological similarity between SS and AT (19-21) has been reported. In previous studies, it was determined that the prevalence of AT in patients with primary SS increased compared with the normal population (5). In another study, it was confirmed that patients with euthyroid AT had increased oxidative modification of both the parotid and submandibular glands and that this was associated with autoimmunity (3).

Animal studies have shown that thyroid dysfunction can affect the secretory unit of the salivary gland (22). The secretory function of the submandibular glands is impaired in patients with AT, as shown by Agha-Hosseini et al (23). Syed et al. (24) showed that there may be significant involvement of the salivary glands in AT cases, that there is a significant decrease in sialometric values in AT patients, and that AT may be the cause of hyposalivation.

Rubaltelli et al. (25) and Nozaki et al. (26) stated that the hypoechoic area is a specific finding in chronic recurrent parotitis and patients with SS. Parenchymal heterogeneity was also noted as another finding. hypoechoic areas on sonograms correlate with findings on sialograms and that US may play an important role in diagnosis.

There is also evidence that hypoechoic areas are not only a sign of peripheral sialectasis but also of lymphocytic infiltration around the ducts (27), and argued that symographic changes could be verified more precisely than sialography. US is very sensitive in the detection of inflammatory changes in the salivary glands but has a lower degree of specificity. We observed that the parenchyma echo changes observed in the US evaluation of the submandibular and parotid glands, which were shown to be functionally affected in AT in previous studies, differed significantly compared with the control group. Although it is known that the echo structure of normal glands in children may vary according to the age and structure of the children, our data on a similar age range in the control group suggest that our findings are not related to the age of the children. In addition, patients with CBC and clinical signs of infection were not included to rule out infectious causes, which are common causes of parenchymal changes. We did not observe a significant difference in Doppler US between the patients and the control group. We believe that quantitative and advanced imaging techniques are required to assess glandular vascularity.

Systemic subclinical inflammation is the cause of comorbidities in children and adults (28,29). While there was no difference in NLR between patients with differentiated thyroid cancer and patients with benign thyroid nodules (30), higher values were associated with tumor size, invasion, and metastasis (31). In other studies, it has been shown that NLR and PLR values are higher in patients with Hashimoto's thyroiditis (32-34). Another study showed that obesity-induced thyroid dysfunction may be associated with inflammatory markers (NLR, PLR, and SII) (35). It has previously been shown that SII can be used in patients with subacute thyroiditis in the diagnosis and follow-up (36,37). In our study, we investigated SII and PIV values as parameters that can predict the salivary gland involvement in children with thyroiditis. We found that both were predictors of salivary gland involvement in children with thyroiditis. In addition, when both parameters were compared using pairwise ROC analysis although the AUC value of the SII parameter was higher, there was no statistical difference. We believe that salivary gland involvement in children with thyroiditis can be predicted from these parameters, which are used as indicators of acute inflammation.

Study Limitations

The main limitations of our study are its single-centre nature, a small number of patients, and cross-sectional design. One of the limitations of our study is that due to its routine use in our clinic, gray scale US and Doppler US were used and no quantitative evaluation was performed. Further studies with advanced US imaging methods are needed.

Conclusion

In our study, we observed that there may be salivary gland parenchymal changes in patients with AT, and SII and PIV inflammatory markers predict these changes, and at this point, the SII value may be more valuable than the PIV value. There is a need for large-scale studies to clarify this issue.

Ethics Committee Approval: The study was approved by the İnönü University Scientific Research and Publication Ethics Committee (approval number: 2020/491, date: 17.03.2020).

Informed Consent: It was obtained.

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Authorship Contributions: Surgical and Medical Practices - S.D.T., A.S., N.Ç., A.C., G.M.D., A.A.; Concept - S.D.T., A.S., N.Ç., A.C., G.M.D., A.A.; Design - S.D.T., A.S., N.Ç., A.A.; Data Collection or Processing - S.D.T., A.S., N.Ç., A.C., G.M.D., A.A.; Analysis or Interpretation - S.D.T., A.S., A.C., G.M.D.; Literature Search - S.D.T., A.S., A.C., G.M.D.; Writing - S.D.T., A.S.

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Accuracy of Blast Enumeration in Bone Marrow by Cytomorphology, Flow Cytometry Immunophenotyping, and Immunohistochemistry Methods in Patients with Myelodysplastic Neoplasm and Acute Myeloid Leukemia

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ABSTRACT

Introduction: Blast percentage (BP) of bone marrow (BM) is crucial for diagnosis, classification, and prognosis in patients with myelodysplastic neoplasms (MDS) and acute myeloid leukemia (AML). Cytomorphology (CM) is considered the gold standard for BM blast counts. Immunohistochemistry (IHC) and flow cytometry immunophenotyping (FCI) methods are used as additional tools. This study aims to compare the overall accuracy of CM, FCI, and IHC in determining BM BP and to evaluate the accuracy of diagnostic and prognostic BP groups determined by these methods in MDS and AML cases.

Methods: CM, CD34-IHC, and FCI were performed on BM biopsy and aspiration samples from MDS and AML patients diagnosed between 9/2019 and 6/2021. Based on BP, cases were divided into four groups: <5%, ≥5%-<10%, ≥10%-<20%, ≥20%. The accuracy of the three methods was statistically compared in terms of blast values and BP groups.

Results: Sixty-eight cases were analyzed. The Pearson-r correlation coefficients for FCI-IHC, CM-FCI, and CM-IHC comparisons were 0.8865, 0.8787, and 0.9670, respectively, suggesting a strong correlation. A comparison of BP groups revealed 86.7%, 79.4%, and 88.2% of the cases in the same blast range by CM-FCI, CM-IHC and IHC-FCI, respectively. Fourteen (20.6%) cases were placed in two different BP groups using different methods.

Conclusion: There was a strong correlation between CM, FCI, and IHC in determining BP; however, one-fifth of the cases were classified differently by three methods, leading to diagnostic and prognostic changes. It is most reliable to combine all three methods taking into account the advantages and disadvantages of each.

Keywords: Bone marrow, blast, cytomorphology, flow cytometry, immunohistochemistry, CD34

Introduction

Myelodysplastic neoplasms (MDS) are a group of clonal hematopoietic stem cell neoplasms defined by cytopenias and morphologic dysplasia characterized by progressively ineffective hematopoiesis and increased risk of acute myeloid leukemia (AML) (1). The term "myelodysplastic syndromes", which was being used in the former editions of World Health Organization (WHO) classifications, has been replaced by "MDS" in the latest edition, which was published in 2022 (1). The annual age-adjusted incidence is approximately 4.0/100,000, and the incidence increases with age (2). Survival is highly variable in MDS patients (3).

Over the years, several classification systems for predicting the prognosis, therapy response, and transformation to AML have been proposed, including the International Prognostic Scoring System (IPSS) and revised IPSS, with revised IPSS being the most up-to-date and widely used (4-7). Studies on large numbers of MDS patients indicated that 34-39% of the cases present with higher risk disease (1,7). The prognostic factors scored in the revised IPSS are the percentage of blast cells in the bone marrow (BM), cytogenetics, hemoglobin concentration, platelet count (blood) and absolute neutrophil count (blood) (7). Thus, the detection of blast percentage (BP) in the BM is crucial in diagnosing AML but also critical in the prognostic classification of MDS.

Three methods can be used to determine the BP in BM: cytomorphology (CM), flow cytometry immunophenotyping (FCI), and immunohistochemical (IHC) examination. In the most recent WHO classification, CM is considered the gold standard for blast counts (1). To

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determine the BP in BM, it is recommended that a 500-cell differential count of all nucleated cells be performed in a smear or trephine biopsy imprint (1). However, blast counts may be hard to appreciate in CM due to low cellularity, BM fibrosis, poor preparation quality, or low observer experience (8-12). Therefore, CD34 IHC analysis is recommended as a method for evaluating the BP in MDS cases (8,13). Other than that, studies report a strong correlation between FCI and CM and state that the addition of FCI to the diagnostic work-up can provide increased accuracy and reproducibility (14-16).

The objective of this study was to evaluate the accuracy of BM BP determined by CM, FCI, and IHC, as well as to evaluate the accuracy of these three methods for diagnosing and predicting BP in cases of AML and myelodysplastic syndrome.

Methods

Case Selection

This study is designed as a retrospective study performed by evaluating BM material including BM trephine biopsy, BM aspirates, and BM aspirate smears of MDS cases, AML cases, and cases diagnosed with AML and receiving bone marrow transplantation (BMT) between 9/2019 and 6/2021 in a single center. For inclusion in the study, BM aspiration samples should be analyzed by CM and FCI, and CD34 IHC should be applied to BM biopsy samples. Regardless of the French-American-British (FAB) classification, all AML cases fulfilling the study inclusion criteria were included in the study. A case lacking at least one of these three studies was excluded from the study. Also, cases with a diagnosis of AML but without CD34-positive blasts were not included in the study. Figure 1 shows the flowchart of the study inclusion and the exclusion process. Ethical approval for this study was obtained from the Yeditepe University Non-Interventional Clinical Studies Ethics Committee (decision number: E.83321821-805.02.03-147, date: 10.02.2023). The study protocol conformed to STARD and ethical guidelines established in the World Medical Association Declaration of Helsinki.

The Evaluation of Cases

In each case; BP was evaluated by CM, IHC, and FCI. Grade of BM fibrosis according to the European Consensus was documented for each case (17). CM was evaluated by a single pathologist. CD34-positive BP was determined by the IHC method in BM trephine biopsies in all cases. CD34 immunohistochemical staining (clone QBEnd/10, Leica Biosystems) was applied to formalin-fixed-paraffin-embedded tissues using an automated staining device (Leica ST5010 Autostainer XL, Leica Biosystems). The percentage of CD34-positive blasts was determined by the FCI method in all cases of BM aspiration. All aspiration samples were processed within 2 hours. Flow cytometry analysis was done with Beckman Coulter Cytomics NAVIOS system and List Mode analysis was done with KALUZA Software Analysis program. According to BP, cases were divided into four groups: <5%, ≥5%-<10%, ≥10%-<20%, ≥20%.

Statistical Analysis

Pearson's correlation coefficient and Spearman's rank correlation coefficient were used to assess the correlation between blast cell percentages determined by the CM, IHC and FCI methods. The accuracy

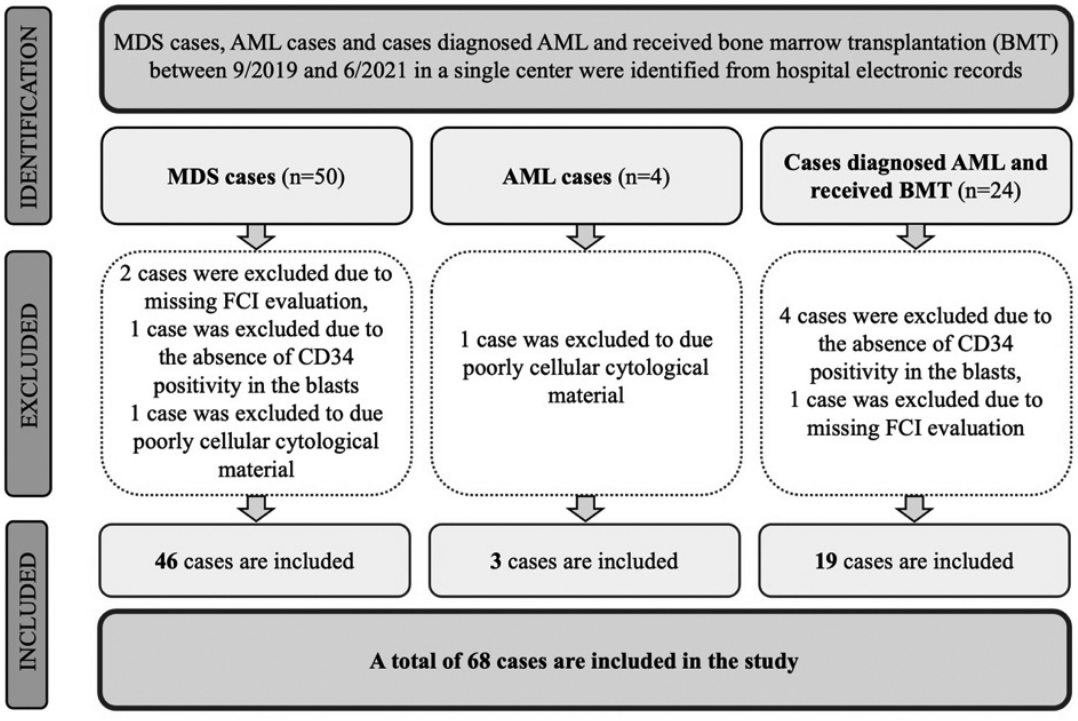

Figure 1. Flowchart of case selection
MDS: Myelodysplastic neoplasms, AML: Acute myeloid leukemia, FCI: Flow cytometry immunophenotyping

of the BP groups determined by each method was compared. The effect of BM fibrosis on the determination of BP groups using different methods was evaluated by Mann-Whitney U test. The statistical significance level was taken as 5% in the calculations. The analysis was conducted using the SPSS (IBM SPSS for Windows, versiyon 26) statistical package program.

Results

A total of 68 cases were analyzed. The cases were composed of 39 MDS, 7 MDS-EB, 3 AML, and 19 cases of AML treated by BMT. Among 22 patients diagnosed with AML, the FAB classification was as follows: 6 AML-M1, 11 AML-M2, 2 AML-M3, 2 AML-M4 and 1 AML-M5. The mean age was 64.6 in MDS cases and 51.2 in AML cases. The female to male ratio was 1.3:1.

In the CM-FCI, CM-IHC, and FCI-IHC comparisons, the Pearson correlation coefficients were 0.8865, 0.8787, and 0.9670, respectively, suggesting a strong correlation. A comparison of CM-FCI cases based on BP groups revealed that 86.7% were in the same blast range (Table 1). Based on the CM-IHC comparison, 79.4% of the cases falls within the same blast range (Table 2). There was an 88.2% correlation between IHC-FCI BP groups based on comparison of the two methods (Table 3). In 79.4% (n=54) of the cases, the BP group determined by all three methods was the same. The remaining 20.6% (n=14) of the cases were placed in two different BP groups using different methods. In these 14 cases, BP groups determined by different methods were consecutive groups. Compared with CM, 5 of these cases (7.4% of all cases) were moved to one upper BP group with either IHC or FCI, whereas 9 cases (13.2% of all cases) were classified in one lower BP group. None of the cases were placed in three different BP groups using three different methods.

BM fibrosis was evaluated in a two-tiered fashion: grade 0-1 and grade 2-3. The cases that were in the same prognostic group by every three methods (n=54) and the cases that were classified differently by different methods (n=14) did not have a significant difference in terms of the presence of marrow fibrosis. The BM fibrosis grades in the first group were as follows: grade 0: 4 cases, grade 1: 12 cases, grade 2: 27 cases, and grade 3: 11 cases. In the second group, the BM fibrosis grades were grade 0: 1 case, grade 1: 3 case, grade 2: 7 case, and grade 3: 3 case. The grade of BM fibrosis did not affect the determination of prognostic groups using different methods (Table 4).

Discussion

Detection of blast rate in the BM is important in the determination of MDS subgroups and in the differential diagnosis of MDS-AML. Also,

Table 1. Comparison of blast percentage groups by CM and FCI

	CD34-positive blasts by FCI				
Blasts by CM	**<5%**	**≥5%-<10%**	**≥10%-<20%**	**≥20%**	**Total**
<5%	53 (77.9%)	1 (1.5%)	0 (0%)	0 (0%)	54 (79.4%)
≥5%-<10%	4 (5.9%)	1 (1.5%)	0 (0%)	0 (0%)	5 (7.4%)
≥10%-<20%	0 (0%)	1 (1.5%)	1 (1.5%)	0 (0%)	2 (2.9%)
≥20%	0 (0%)	1 (1.5%)	2 (2.9%)	4 (5.9%)	7 (10.3%)
Total	57 (83.8%)	4 (5.9%)	3 (4.4%)	4 (5.9%)	68 (100%)

CM: Cytomorphology, FCI: Flow cytometry immunophenotyping

Table 2. Comparison of blast percentage groups by CM and IHC

	CD34-positive blasts by IHC				
Blasts by CM	**<5%**	**≥5%-<10%**	**≥10%-<20%**	**≥20%**	**Total**
<5%	50 (73.5%)	4 (5.9%)	0 (0%)	0 (0%)	54 (79.4%)
≥5%-<10%	5 (7.4%)	0 (0%)	0 (0%)	0 (0%)	5 (7.4%)
≥10%-<20%	0 (0%)	2 (3%)	0 (0%)	0 (0%)	2 (3%)
≥20%	0 (0%)	0 (0%)	3 (4.4%)	4 (5.9%)	7 (10.3%)
Total	55 (80.9%)	6 (8.8%)	3 (4.4%)	4 (5.9%)	68 (100%)

CM: Cytomorphology, IHC: Immunohistochemistry

Table 3. Comparison of blast percentage groups by IHC and FCI

	CD34-positive blasts by FCI				
CD34-positive blasts by IHC	**<%5**	**≥5%-<10%**	**≥10%-<20%**	**≥20%**	**Total**
<5%	53 (77.9%)	2 (2.9%)	0 (0%)	0 (0%)	55 (80.8%)
≥5%-<10%	4 (5.9%)	1 (1.5%)	1 (1.5%)	0 (0%)	6 (8.9%)
≥10%-<20%	0 (0%)	1 (1.5%)	2 (2.9%)	0 (0%)	3 (4.4%)
≥20%	0 (0%)	0 (0%)	0 (0%)	4 (5.9%)	4 (5.9%)
Total	57 (83.8%)	4 (5.9%)	3 (4.4%)	4 (5.9%)	68 (100%)

IHC: Immunohistochemistry, FCI: Flow cytometry immunophenotyping

Table 4. Comparison of the BM fibrosis grades of the cases that are placed in the same BP group by three methods and those that are placed in different BP groups by different methods

BM fibrosis grade	Cases placed in the same blast percentage group using three methods (n=54) (%)	Cases placed in different blast percentage groups using different methods (n=14) (%)
Grade 0	4 (7.5%)	1 (7.2%)
Grade 1	12 (22.2%)	3 (21.4%)
Grade 2	27 (50%)	7 (50%)
Grade 3	11 (20.3%)	3 (21.4%)

BM: Bone marrow, BP: Blast percentage

percentage of blast cells in the BM is among the prognostic factors scored in the revised IPSS, a standard for assessing the prognosis of primary untreated adult patients with MDS (7). This increases the importance of accurately determining the blast rate in the BM.

In this study, a strong correlation was observed for absolute values of blasts with CM, IHC, and FCI evaluation methods. Almost 80% of the cases were placed in the same BP group using three methods. However, in 20% of the cases, the BP group was determined differently by different methods, leading to a change in the diagnostic and prognostic blast group of the cases. BM fibrosis grade did not show any effect in determining the prognostic groups by different methods.

CM is considered the gold standard for blast counts in BM. Cytomorphological assessment relies heavily on the quality of the cytological smears and the experience of the observer, which makes the reproducibility of this method limited in some circumstances. Low cellularity, BM fibrosis, poor preparation quality, and low observer experience have been shown to have an effect on cytomorphological blast counts (8-12). CM is shown to be a reliable method in blast counting with respect to the 5% cut-off; however, the reproducibility of the blast counts decreases under the 5% limit, especially under 2% (10,11). Also, it has been reported that smears taken from patients with hypoplastic MDS or MDS with fibrosis greater than WHO grade 2 can show low cellularity, and the blast count may not be fully representative in these cases (8,18,19).

The drawbacks of CM in determining blast cell percentage make immunohistochemical analysis and flow cytometry analysis with CD34 especially helpful when there is hypocellular BM or fibrosis, which often causes underestimation of BP in smears (13). Another important point is identifying CD34-positive cell clusters by IHC in the BM, which are reported to have prognostic importance for progression to AML (20,21). The European LeukemiaNet recommends the evaluation of CD34-positive cells in BM biopsy as a standard diagnostic tool in the approach to MDS (22). As for the use of FCI in the evaluation of BP in BM, while some studies show that FCI detects a lower number of blasts than CM (23,24), newer studies report a strong correlation between FCI and CM (14-16). In their multicenter study, Font et al. (25) investigated the reproducibility of CD34-positive cell count by FCI. They found an overall excellent agreement on CD34-positive cell count among participants and concluded that the standardization of routine flow cytometry laboratory practices is mandatory (25). In a more recent study, Johansson et al. (26) reported excellent concordance between seven cytometrists in myeloid progenitor cell count, concluding that FCI offers a reliable diagnostic and prognostic measurement in MDS patients.

Previous studies have shown varying results regarding the correlations between CM-IHC and CM-FCI. Hodes et al. (27) assessed the accuracy of blast cell quantification in 16 BM samples containing varying numbers of CD34-positive blasts. There was a poor correlation (R^2=0.52) between CM BP and trephine biopsy BP stained with CD34. A good correlation was demonstrated between the number of blasts determined by FCI and IHC (R^2=0.81). Recent research by Saft et al. (28) determined CD34+ blast counts in 132 BM biopsies by IHC and compared them with those obtained by CM and FCI. In the CM-IHC comparison, Pearson's r correlation was 0,728 and in the CM-FCI comparison, Pearson's r correlation was 0,782. The correlation between CM-IHC and CM-FCI was slightly higher in this study than in the study by Saft et al. (28); which may be attributable to the difference between case numbers. Their study also found that 17% of patients had higher blast counts by IHC compared to CM, which affected subclassification in MDS. However, in our study 7.4% of the cases were moved to one upper BP group with either IHC or FCI, whereas 13.2% of all cases were classified in one lower BP group. This difference highlights the importance of material adequacy, preparation quality, and interobserver variability in CM.

Study Limitations

There is a limitation to this study in that only CD34-positive cases were included; however, it is known that not all blasts express CD34. In addition, future studies implementing clinical and survival data will be very useful in determining the most accurate approach in this regard.

Conclusion

An overall strong correlation was observed between CM, IHC, and FCI in determining the BM blast counts in MDS and AML. In one-fifth of the cases, the BP groups determined by the three methods were different, and this difference led to diagnostic and prognostic classification changes. Therefore, the combination of these three methods will be the most accurate and reliable approach in terms of patient management when determining the BM BP that has diagnostic and prognostic importance.

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Informed Consent: Retrospective study.

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Authorship Contributions: Surgical and Medical Practices - E.H.; Concept - E.H., F.Ö.; Design - E.H., F.Ö.; Data Collection or Processing - E.H.; Analysis or Interpretation - E.H., F.Ö.; Literature Search - E.H.; Writing - E.H.

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Endotheliopathy, Soluble Thrombomodulin and Its Role in Predicting Prognosis in Severe Coronavirus Disease-2019 Pneumonia

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ABSTRACT

Introduction: The main reason for acute respiratory distress syndrome (ARDS) and mortality during coronavirus disease-2019 (COVID-19) infection is thrombotic events due to the tendency to coagulopathy. Systemic inflammation, endothelial dysfunction caused by severe hypoxia and thrombocyte abnormalities lead to coagulopathy. Here, we aimed to search for the relationship of soluble thrombomodulin (TM) and von Willebrand factor (vWF) antigen levels as endothelial dysfunction biomarkers with an early stage severe COVID-19 infection.

Methods: Fifty-four patients admitted to our hospital with severe COVID-19 infection and 25 healthy asymptomatic patients were included in the study. Both the patient (at hospital admission date) and healthy control group gave venous blood samples for soluble TM and vWF antigen level measurements. The level of the searched parameters were compared between groups and hospital admission duration and mortality rate of the patient group. Results were evaluated using the SPSS program.

Results: vWF antigen levels did not show any difference between groups, but soluble TM was significantly higher in the patient group. Thus, soluble TM level did not show a statistically significant relationship with duration of hospitalization or mortality.

Conclusion: Early elevation of soluble thrombolic level can be considered as an early defense mechanism of endothelium against thrombosis in a severe COVID-19 infection.

Keywords: Solubl thrombomodulin, von Willebrand factor, COVID-19 infection

Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), also called as coronavirus disease-2019 (COVID-19) infection, raised from China, city of Wuhan in 2019 and spread globally in a short time to cause a pandemic that resulted in millions of people to get infected and so many to die. The most important feature of the virus was rapid contamination and the respiratory system was the most affected system. Its infection ranges from asymptomatic infection/mild respiratory infection to severe pneumonia and acute respiratory distress syndrome (ARDS) (1).

Severe hypoxia and inflammation set the ground for thrombotic diseases. Venous thromboembolic was seen in hospitalized patients between 16-49% (2-4). Thrombotic complications present in a wide range from pulmonary emboli to macrothrombosis in large arteries and veins and are accepted as a poor prognostic predictors (5-14). The virus is known to directly invade the alveolar epithelium to enter the body and cause a cytopathic effect on the alveolar epithelium, but it is not shown directly in the endothelium. Extensive endothelium dysfunction causes microthrombosis (15).

Thrombomodulin (TM) is a thrombin receptor with high affinity present in the endotelium membrane and presents as a natural anticoagulant. It acts as a cofactor of protein C thrombin - catalyzed activation and inhibits thrombin's procoagulant functions. Endothelial TM enzymatically splits in the presence of cytokines, active neutrophils, and macrophages. It releases soluble fragments that circulate in blood and are excreted urinally. These soluble parts are called soluble TMs. TM level is set as a molecular biomarker that reflects the damage of endothelial cells (16,17). vWF is a platelet - adhesive protein and the carrier of coagulation factor VIII synthesized by endothelial cells and megakaryocytes (18). The role of increased vWF in the prothrombotic picture in the course of COVID-19 has been demonstrated.

Here, we aimed to search for the level and role of soluble TM and vWF antigen levels as endothelial damage indicators and endothelial dysfunction biomarkers in early stage severe COVID-19 infection.

Methods

Ethical Statement

The protocol for this study was approved by the Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2800, date: 02.04.2021). All included patients

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provided written informed consent for the publication of their medical details.

Study Design and Participants

The study was designed as a cross-sectional case-control study. 54 patients (29 F, 34 M) admitted to our hospital with severe COVID-19 infection and 25 (F: 11, M: 14) healthy asymptomatic patients were included in the study. Clinical and laboratory features of all patients were recorded.

Both the patient (at hospital admission date) and healthy control group gave venous blood samples for soluble TM and vWF antigen level measurements. The level of the searched parameters were compared between groups and hospital admission duration and mortality rate of the patient group.

Severe COVID-19 pneumonia was described according to clinical, radiological, and laboratory criteria for severe COVID-19 pneumonia according to the Living Guidance for Clinical Management of COVID-19 (19). COVID-19 infection was confirmed from oropharyngeal and nasal swap samples by real-time reverse transcriptase polymerase chain reaction testing. After admission to the hospital, venous blood samples were collected using standard blood sampling and laboratory methods and sent to the laboratory. Blood samples were centrifuged at 1500 rpm in tubes with EDTA and kept in -80 °C. Blood samples were tested for soluble TM and vWF antigen levels. Inflammatory parameters [C-reactive protein (CRP), ferritin, procalsitonin] were also tested. The effect of these values on the duration of hospitalization and mortality rates was evaluated. The same blood parameters were also measured in the healthy control group and compared with the patient group statistically.

Statistical Analysis

Statistical analysis was performed using SPSS 28.0 for the Windows program. $P<0.05$ was considered statistically significant. Descriptive statistics were reported as the mean, standard deviation, median, minimum, maximum, frequency, and percentage values. Quantitative independent data analysis was performed with independent sample t-test and Mann-Whitney U test. Qualitative independent data analysis was conducted with the chi-square test and the Fisher's test when this was not applicable. The effect size and cutoff values were evaluated with receiving operating characteristic analysis.

Results

Mean age was 53.2±9.1 years (F: 24/M: 35) in the patient group and 48.2±10.4 years (F: 11/M: 14) in the control group. In the patient group with accompanying diseases, 24 patients (40%) had hypertension 12 patients (20%) had diabetes mellitus, and 4 patients (6%) had NHYA 1-2 heart failure mean duration of hospitalization was 8 days (4-41). Demographic and clinical features of the patients are summarized in Table 1.

There was no statistically significant difference between the patient and control groups for orgender distribution ($p>0.05$). The mean vWF

Table 1. Laboratory, clinical and demographic characteristics of patients

		Min.-Max.	Median	Mean ± SD/(n, %)
Age		30.0-70.0	53.0	51.7±9.7
Sex	Female			24 (41.7%)
	Male			35 (58.3%)
Comorbidity	(-)			30 (50.8%)
	(+)			29 (49.2%)
Hypertension				24 (40.7%)
Diabetes mellitus				12 (20.3%)
Cardiovascular disease				4 (6.8%)
vWF, ng/mL		1.9-54.3	40.3	37.8±12.0
Trombomodulin, ng/mL		3.1-10.1	5.1	5.2±1.0
Haemoglobin, g/L		8.8-15.3	13.0	12.7±1.3
Urea		12.0-111.0	25.0	29.8±17.4
Creatinine, µmol/L		0.1-2.2	0.7	0.8±0.3
CRP (mg/L)		1.0-229.0	30.0	57.4±62.0
Procalcitonin, ng/mL		0.0-1.3	0.1	0.1±0.2
Ferritin, pg/dL		10.0-1500	147.5	284.0±334.8
Aspartate aminotransferase, U/L		12.0-195.0	33.0	37.6±28.1
Alanine aminotransferase, U/L		6.0-142.0	26.0	33.3±24.0
Lactate dehydrogenase, U/L		0.0-803.0	251.0	271.4±126.5
Creatine kinase, U/L		15.0-1049.0	69.5	135.0±175.7
Prognosis	Discharge			52 (88.1%)
	Death			7 (11.9%)
Hospitalization (day)		4.0-41.0	8.0	9.5±6.5

Min.: Minimum, Max.: Maximum, SD: Standard deviation, vWF: von Willebrand factor, CRP: C-reactive protein

antigen level was 37.8 ng/dL in the patient group and 44.3 ng/dL in the control group, and there was no significant difference between 2 groups (p>0.05). There was no difference in Hb and creatinine levels between the groups (p>0.05). In the patient group, TM, CRP, procalsitonin, ferritin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase levels were significantly higher than those in the control group (p<0.05) Table 2.

In different patient and control groups, TM level was found to have a significant effect [area under the curve (AUC): 0.765 (0.650-0.880)]. The 5 cut-off value for TM was found to be effective to differentiate patient and control groups [AUC: 0.739 (0.623-0.855)]. Sensitivity 67.8%, positive prediction 88.9%, specificity 80.0%, negative prediction 51.3% Table 3.

Discussion

This study showed that in early stage severe COVID-19 solubl TM level was increased while vWF level did not show a significant change. These findings can be interpreted as in early stages of severe COVID-19 infection endothelial activation developed and this feature provided defense and protection against thrombosis.

Studies have shown that abnormal coagulation parameters are related to poor prognosis and fibrin degradation products, and D-dimer levels are predictors of mortality in COVID-19 infection (20). This opinion was supported when a decrease in mortality was seen after heparin had been used during the disease (21).

The endothelium has some physiological functions such as vascular tonus control, tissue homeostasis, barrier integrity, anti-inflammatory and antioxidant effects, vascular permeability regulation, structural and vascular integrity, and prevention of thrombosis (22). Endothelitis and endothelium dysfunction and accompanying platelet abnormalities that occur during COVID-19 infection cause a tendency to thrombosis and affect the prognosis of the disease.

Goshua et al. (23) showed in their study that in patients admitted to intensive care unit (ICU), vWF antigen levels were increased when compared to on-ICU patients. Solubl TM level was higher than 3.26 ng/mL and this level was negatively correlated with hospital discharge. Also, vWF vs solubl TM levels were related to mortality (23). It is known that in late stages of the disease, severe hypoxia and systemic inflammation cause endothelium dysfunction and a tendency to thrombosis (24). In

Table 2. General features and laboratory values of case and control groups

Mean ± SD/(n, %)		Control group		Case group		p	
		Median	Mean ± SD/(n, %)	Median			
Age (year)		48.2±10.4	51.0	53.2±9.1	54.0	0.052	t
Sex	Female	11 (44.0%)		24 (40.7%)		0.778	X^2
	Male	14 (56.0%)		35 (59.3%)			
vWF (ng/mL)		42.5±6.4	44.3	35.8±13.2	37.8	0.066	m
Trombomodulin (ng/mL)		4.6±0.8	4.5	5.4±1.0	5.5	**0.001**	t
Haemoglobin, g/L		12.9±1.1	13.0	12.7±1.4	12.9	0.425	t
Urea		23.8±15.9	20.0	31.5±17.5	26.5	**0.004**	m
Creatinine, µmol/L		0.72±0.20	0.70	0.79±0.33	0.80	0.544	m
CRP (mg/L)		3.8±2.4	4.0	80.1±61.1	61.0	**0.001**	m
Procalcitonin, ng/mL		0.01±0.01	0.02	0.11±0.18	0.08	**0.001**	m
Ferritin, pg/dL		60.9±37.7	54.0	378.5±359.6	269.0	**0.001**	m
Aspartate aminotransferase, U/L		22.9±9.9	22.0	44.0±30.9	37.5	**0.001**	m
Alanine aminotransferase, U/L		23.0±10.1	22.0	37.7±26.9	29.0	**0.005**	m
Lactate dehydrogenase, U/L		166.9±43.1	150.0	316.5±124.0	299.5	**0.001**	m
Creatine kinase, U/L		53.9±22.6	54.0	170.6±200.5	114.0	**0.001**	m

t: T-test, m: Mann-Whitney U test, X^2: Chi-square test, vWF: von Willebrand factor, CRP: C-reactive protein, SD: Standard deviation

Tablo 3. Predictive value of thrombomodulin with receiving operating characteristic analysis

		Area under the curve		CI 95%	p
Thrombomodulin, (ng/mL)		0.765		0.650-0.880	**0.001**
Thrombomodulin, cut-off 5		0.739		0.623-0.855	**0.001**
		Control	Case		
Thrombomodulin, (ng/mL)	≤5	20	19	Sensivity	67.8%
	>5	5	40	Positive prediction	88.9%
				Specificity	80.0%
				Negative prediction	51.3%

CI: Confidence interval

postmortem examination, in pulmonary endothelium cells of patients who died because of COVID-19 infection, TM expression was found to be decreased and vWF expression was increased. The increased number of immun cell infiltration was thought to decrease TM levels (25).

Decreased oxygenization, which means hypoxia is a direct risk factor for thrombosis (26,27). Hypoxia stimulates some cell signal pathways to initiate thrombosis and also suppresses TM, which is an anticoagulant (28-30). Here, it should be considered that TM is increased in the early stages of the disease while it is decreased in the advanced period and contributes to thrombosis. In our study, the increase in TM at early stages can be explained as the defense of endothelial cells against thrombosis. This finding can lead the way for treatment. Studies showed that replacement of recombinant soluble TM in septic patients with disseminated intravascular coagulation decreased mortality (24,31,32). TM is an endothelium function biomarker that has anticoagulant as well as anti-inflammatory effects immune cell adhesion and complement system activation (16,17).

Our study showed that vWF antigen levels did not have a significant difference in patients compared with healthy controls. This could be explained by the early stages of the disease. In different studies, vWF antigen levels were shown to be increased in the advanced stages (21st day) of the disease parallel to an increase in factor-8 levels. This was described as strong stimulation and damage of the endothelium and increase of vWF levels in Weibel-Palade bodies (33). vWF levels were found to be higher in ICU patients than in non-ICU patients and was related to mortality (23). This is also supported by another study (34-36).

This shows that vWF-level increases with duration and severity of the disease, contributes to thrombosis and mortality in critically ill patients.

In COVID-19 infection first target is alveolar target and there is no evidence showing that virus directly infects the endothelium and this was supported with postmortem studies (25,37-39). But there are limited number of studies contradicting this idea showing that decreased angiotensin-converting enzyme-2 expression caused decreased endothelium cell sensitivity to SARS-CoV-2 (40-46). Phenotypic changes of endothelium in COVID-19 infection are not enlightened yet but studies showed that endothelial cell activation and damage played an important role in ARDS, pulmonary edema and procoagulant stage related COVID-19 pathogenesis (22,47).

Study Limitations

The small number of patients in our study is a limitation of the study.

Conclusion

It can be considered that an increase in soluble TM in early stages of COVID-19 infection has a protective effect against thrombosis, but in advanced stages, a decrease in TM due to endothelial dysfunction plays an active role in thrombosis development and negatively affects prognosis of the disease.

Ethics Committee Approval: The protocol for this study was approved by the Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2800, date: 02.04.2021).

Informed Consent: All included patients provided written informed consent for the publication of their medical details.

Peer-review: Externally peer-reviewed.

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Wnt1 and β-Catenin Expression in Lobular Capillary Hemangioma: Immunohistochemical Study

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ABSTRACT

Introduction: Lobular capillary hemangioma (LCH) is a vascular neoplasia with a pathogenesis that has not been fully clarified. The Wnt signaling pathway plays an essential role in vasculogenesis and angiogenesis. The increased activity in the Wnt signaling pathway may cause some neoplastic proliferations. This study revealed the role of the Wnt1/β-catenin signaling pathway in the development of LCH.

Methods: The study included 30 LCH tissue samples. Vascular structures in healthy tissue surrounding LCH lesions were used as controls. Wnt1 and β-catenin protein expressions in tumor tissue and surrounding tissue vascular structures were evaluated using the immunohistochemical method.

Results: Wnt1 expression was 1.10±0.16 and 0.33±0.10 in the LCH and control groups, respectively, indicating a statistically significant difference ($p<0.001$). β-catenin expression was 1.57±0.39 and 0.59±0.18 in the LCH and control groups, which was also statistically significant ($p<0.001$). Wnt1 and β-catenin expressions were similar in the mucosal and cutaneous LCH lesions ($p>0.05$).

Conclusion: In LCH, the Wnt1/β-catenin signaling pathway expression is increased. This increase may be due to the increased *Wnt1* gene expression, pathologies in different signaling pathways, or paracrine factors secreted from the tumor microenvironment. Treatment modalities targeting the Wnt1 signaling pathway may be promising for treating LCH.

Keywords: β-catenin, hemangioma, immunohistochemistry, pyogenic granuloma, Wnt β-catenin signaling pathway

Introduction

Lobular capillary hemangioma (LCH), a prevalent type of vascular lesion that affects the skin and mucous membranes, commonly emerges in young adults and children and affects both genders uniformly. Until recently, LCH was considered a vascular proliferation reactive to certain predisposing factors, including chronic trauma, inflammation, and hormonal factors. However, as knowledge of vascular proliferations and vascular tumor pathogenesis increases, the idea that LCH is a true neoplasia is becoming more commonly accepted (1).

During embryonic development, the Wnt signaling pathway is responsible for cell patterning, polarity and migration, cell fate determination, and regeneration of organs and tissues. Besides, there is increasing evidence that Wnt has a significant impact on angiogenesis and vasculogenesis (2). The Wnt family consists of 19 glycoproteins. These proteins use canonical or non-canonical pathways, depending on whether they use β-catenin as a downstream effector. In the canonical Wnt pathway, when Wnt is not present, a protein complex consisting of axin, adenomatous polyposis coli, and glycogen synthase kinase-3β (GSK3β) phosphorylates the cytosolic β-catenin. Upon Wnt proteins binding to Frizzled (Fz) proteins in the presence of coreceptor LDL-receptor-associated protein 5 or 6, the phosphorylation complex containing GSK3β is inhibited, and cytosolic β-catenin accumulates and then β-catenin translocates from the cytoplasm into the nucleus. Following this translocation, β-catenin interacts with transcription factors from the T-cell factor/lymphocyte enhancing factor family and modulates the expression of multiple target genes (2). The impairment in the Wnt signaling pathway can cause developmental defects, degenerative diseases, and some cancers. In the development of infantile hemangioma, one of the vascular tumors, the canonical Wnt pathway activity is increased. Similarly, an increase in β-catenin expression has been reported in pulmonary sclerosis hemangiomas (3,4).

Wnt1 in the chromosome 12q13 region is a member of the Wnt family that uses the canonical signaling pathway. *Wnt1* gene transfection into human umbilical vein endothelial cells has shown that it causes hyperproliferation in endothelial cells (5). In addition, the increased

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Wnt1 expression has been reported in some tumors, such as melanoma, breast cancer, and basal cell carcinoma (6).

In this study, we aimed to elucidate the involvement of the Wnt1/β-catenin signaling pathway in the pathogenesis of LCH.

Methods

In this study, we recruited thirty patients diagnosed with LCH based on histopathological analysis of a skin biopsy performed at Fırat University between March 2019 and May 2021. Patients who had previously undergone cryotherapy, electrocautery, surgical intervention for the lesions, and those who had received any topical treatment were not included in the study. Normal vascular structures in healthy tissues surrounding the lesions were used as the control group. The study was approved by the Fırat University Non-Interventional Research Ethics Committee (approval number: 3956, date: 28.09.2021). Because the samples were obtained from the archives of the Fırat University Faculty of Medicine, Department of Pathology, patient consent was not required.

Immunohistochemistry

Paraffin-embedded specimens were sectioned into 4-micrometer slices and mounted on glass slides coated with Poly-L-Lysine. To retrieve antigens, specimens were deparaffinized and then rehydrated through a series of graded ethanol solutions. Afterwards, the specimens were subjected to microwave boiling in citrate buffer for 12 minutes (7+5) at a pH of 6.0. After cooling the specimens to room temperature for about 20 min, they were subjected to three washes with phosphate-buffered saline (PBS) (P4417; Sigma-Aldrich, St. Louis, MO) for 5 min each. Afterwards, the specimens were incubated with hydrogen peroxide for 5 min to prevent endogenous peroxidase activity (Hydrogen Peroxide Block, TA-125-HP; Lab Vision Corp., New York, NY). Ultra V Block solution (TA-125-UB; Lab Vision Corp.) was applied to the specimens for 5 min. The primary antibodies WNT1 Rabbit pAb (WH1158730; Cat. no: A2475; AB Clonal) and β-catenin (E-5) sc-7963 [mouse monoclonal immunoglobulin G (IgG); Santa Cruz Biotechnology) were diluted at 1:200 and then incubated with the specimens in a humid environment at room temperature for 60 min. Following primary antibody incubation, the specimens were washed three times with PBS for 5 min and subsequently exposed to biotinylated goat anti-polyvalent secondary antibody (anti-mouse/rabbit IgG) (TP-125-BN; Lab Vision Corp.) for 30 min at room temperature. The sections were rinsed thrice with PBS, followed by incubation with streptavidin peroxidase (TS-125-HR; Lab Vision Corp.) for 30 min, and then placed in PBS. A solution of 3-amino-9-ethyl carbazole (AEC) substrate and AEC chromogen (AEC substrate, TA-015-HAS; AEC Chromogen, TA-002-HAC; Lab. Vision Corporation) was used to stain the specimens with AEC. When immunoreactivity became visible under light microscopy, the sections were washed with PBS. Counterstaining was performed using Mayer's hematoxylin. After rinsing the sections with PBS and distilled water, they were covered with a mounting medium (Large Volume Vision Mount, TA-125-UG; Lab Vision Corp.). The Zeiss Axio Scope A1 microscope (Zeiss, Gottingen, Germany) was used to examine and photograph the mounted sections. All samples were scored blindly by a pathologist and histologist. For immunohistochemical scoring, the histological score was calculated as follows: The staining distribution was evaluated using a scoring system where 0.1 represented less than 25%, 0.4 represented 26-50%, 0.6 represented 51-75%, and 0.9 represented 76-100%. Another scoring was used to assess the staining intensity, where 0 indicated no staining, 0.5 indicated very little staining, 1 indicated little staining, 2 indicated moderate staining, and 3 indicated very strong staining. Histoscores were obtained by multiplying the distribution score and intensity score according to the formula histoscore = distribution × intensity (7).

Statistical Analysis

Statistical Package for the Social Sciences (IBM Corp., Armonk, NY, version 25.0) was used for statistical analysis. Numerical variables are presented as mean ± standard deviation (SD) values. On the other hand, categorical variables were described using frequencies and percentages. Parametric distribution of the variables was assessed via the Kolmogorov-Smirnov test. For intergroup comparisons, numerical variables were analyzed using the Student's t-test and Mann-Whitney U test, while categorical variables were evaluated using the chi-square and Fisher's exact (two-sided) tests. Statistical significance was defined as a p-value of less than 0.05.

Results

General Characteristics of the Subjects

Among the 30 patients, an equal number of individuals (n=15) were male and female. The patients had a mean age of 33±21.1 years (mean ± SD). The means macroscopic size of the LCH lesions was 11±6.07 mm. Ten (33%) lesions showed mucosal localization. Of all the lesions, 17 (57%) were observed in the head and neck, seven (23%) in the upper extremity, five (17%) in the lower extremity, while only one (3%) was found on the trunk. Comparisons between genders revealed no statistically significant differences with respect to mean age, lesion size, and mucosal involvement (p>0.05). Table 1 displays a summary of the patients' demographic characteristics (Table 1) (Figure 1).

Table 1. Summary of the patients' clinical data

Characteristic	Female	Male	Total	p-value
Number	15 (50%)	15 (50%)	30 (100%)	
Age, mean (SD, min.-max.)	36 (20, 14-67)	32 (22, 1-67)	33 (21, 1-67)	0.568
Size (mm)	11.33±5.52	10.67±6.77	11.00±6.07	0.770
The mucosal involvement, n (%)				
Yes	7 (47%)	3 (20%)	10 (33%)	
No	8 (53%)	12 (80%)	20 (67%)	
Total	15 (100%)	15 (%)	30 (100%)	0.121

SD: Standard deviation, min.: Minimum, max.: Maximum

Wnt1 Immunoreactivity

Wnt1 expression was 1.10±0.16 in the LCH lesions and 0.33±0.10 in the healthy vascular structures in the tissue surrounding the lesions evaluated as controls, and a significant difference was found between these groups (p<0.001) (Table 2). Wnt1 immunoreactivity was nuclear and cytoplasmic in the vascular endothelial cells, and increased Wnt1 expression was observed in tumor stroma (Figure 2). The presence or absence of mucosal involvement did not yield a statistically significant difference in Wnt1 expression (p>0.05). Wnt1 expression was similar in both genders in the LCH and control groups. The analysis revealed no significant difference in Wnt1 expression with respect to lesion localization (p>0.05) (Table 3).

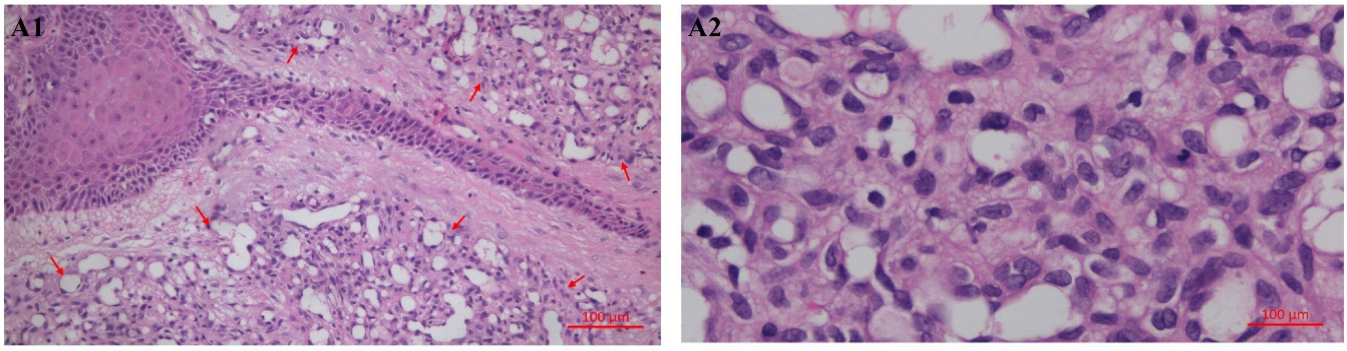

Figure 1. Histopathological examination of the lobular capillary hemangioma (hematoxylin and eosin). Lobular proliferation of capillary-sized vessels within the superficial dermis and mixed inflammatory infiltrates resembling the granulation tissue in the stroma. The infiltration consists of fibroblasts, lymphocytes, neutrophils, plasma cells, and mast cells (A1, A2)

Table 2. Wnt1 and β-catenin immunoreactivity of the study groups

Histoscores of the groups, mean (SD)	Control	LCH	p-value
Wnt1	0.33±0.10	1.10±0.16	**<0.001**
β-catenin	0.59±0.18	1.57±0.39	**<0.001**

SD: Standard deviation; LCH: Lobular capillary hemangioma

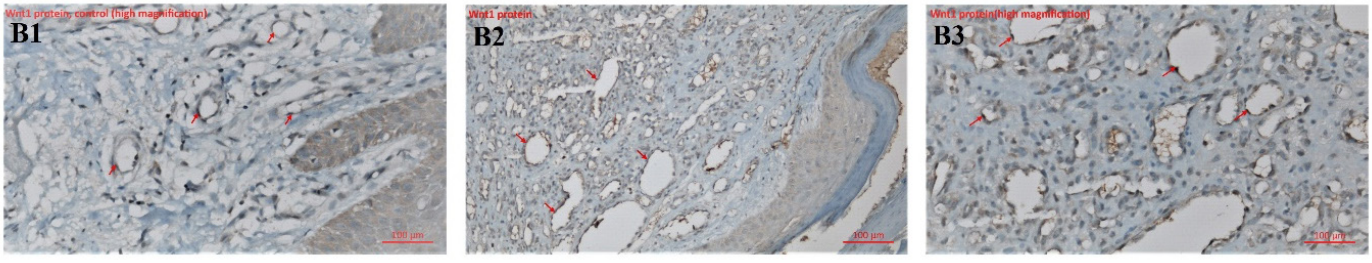

Figure 2. Immunohistochemical analysis of Wnt1 protein. Normal vascular structures in healthy skin adjacent to LCH (B1), vascular structures in an LCH lesion (B2, B3)

LCH: Lobular capillary hemangioma

Table 3. Histoscore of Wnt1 and β-catenin immunoreactivity of the study groups

Histoscore of groups, mean (SD)	Wnt1		β-catenin	
	Control	LCH	Control	LCH
Gender				
Female	0.35±0.11	1.10±0.15	0.57±0.72	1.56±0.36
Male	0.30±0.09	1.10±0.19	0.60±0.20	1.58±0.43
	p=0.124	p=0.795	p=0.741	p=0.802
Mucosal involvement				
Present	0.31±0.84	1.11±0.14	0.555±0.213	1.590±0.414
Absent	0.34±0.11	1.10±0.18	0.600±0.169	1.530±0.359
	p=0.0.547	p=0.818	p=0.533	p=0.700

SD: Standard deviation, LCH: Lobular capillary hemangioma

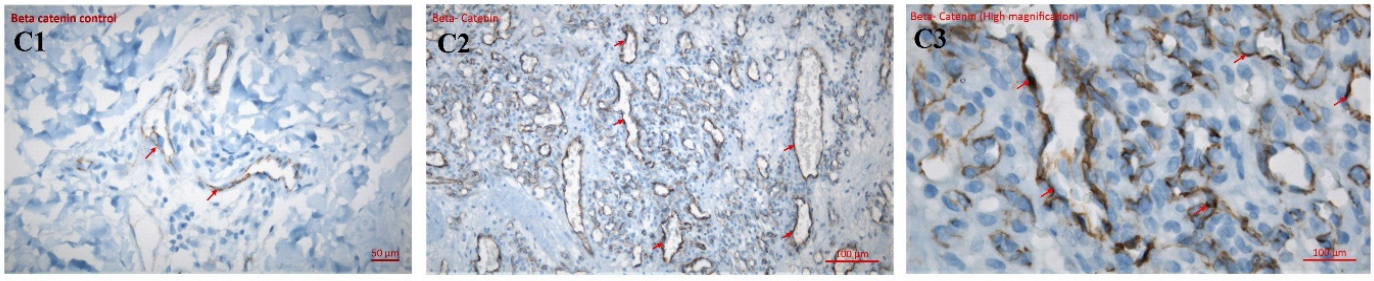

Figure 3. Immunohistochemical analysis of β-catenin protein. Normal vascular structures in healthy skin adjacent to LCH (C1), vascular structures in an LCH lesion (C2, C3)
LCH: Lobular capillary hemangioma

β-catenin Immunoreactivity

β-catenin expression was 1.57±0.39 in the LCH lesions and 0.59±0.18 in the controls, indicating a statistically significant difference ($p<0.001$) (Table 2). β-catenin immunoreactivity was nuclear and cytoplasmic in the vascular endothelial cells. There was no increase in β-catenin expression in tumor stroma (Figure 3). The expression of β-catenin showed no significant differences between lesions with and without mucosal involvement, between male and female patients in both the LCH and control groups, or among different lesion localizations ($p>0.05$) (Table 3).

Discussion

Until recently, LCH, namely pyogenic granuloma, was considered to be reactive vascular proliferation due to its emergence in areas of previous trauma or chronic irritation, increased frequency in some hormonal changes such as pregnancy, presence of Gram-positive (+) and Gram-negative (-) bacilli in microscopic examinations, and association with the use of some drugs (8). However, in recent comprehensive studies, trauma history was observed in only 4.5-15% of the patients, and the observed bacilli were elements of the flora and not found to be a predisposing factor in most patients (9,10). First, Pagliai and Cohen (10) proposed that pyogenic granuloma was a benign acquired vascular neoplasia and used the term "LCH" to describe this condition. In previous studies, hemangioblastic blood islands were observed within LCH lesions, which were observed to contain hematopoietic stem cells (11). Blackwell et al. (12) reported that in LCH, the embryonic stem cell markers OCT4, SOX2, pSTAT3 and NANOG revealed increased expression. They suggested that LCH originated from the primitive endothelium, similar to infantile hemangioma and developed because of *de novo* vasculogenesis (12).

The Wnt pathway has been recognized for its significant involvement in vessel remodeling, angiogenesis, and vasculogenesis. Wnt2, Wnt5a, Wnt7a, and Wnt10b were found to be expressed in endothelial cells, while Wnt-5a was found to be expressed in vascular smooth muscle cells (13). Transfection of the *Wnt1* gene into human umbilical vein endothelial cells increases endothelial cell proliferation (5).

Wnt signaling pathway activation is also known to increase cell proliferation and cause some cancers such as skin melanoma, basal cell carcinoma, colorectal carcinoma, squamous cell carcinoma, and hematological malignancies (2,6,14). It has been suggested that in addition to supporting an epithelial-mesenchymal transition, thereby promoting the maintenance of cancer stem cells, Wnt also increases immune tolerance and limits antitumor response by acting as a bridge between the tumor microenvironment and tumor cells (15). Stephenson et al. (4) showed that infantile hemangiomas, the most common vascular tumors, were derived from stem cells, and Wnt/β-catenin transcription activity was essential for this process, with the inhibition of this pathway also inhibiting the expression of stem cell factors (4). Another study that investigated the Axin, C-myc, and β-catenin expressions in pulmonary sclerosis hemangioma, another vascular tumor originating from the primitive respiratory epithelium, high cytoplasmic β-catenin and C-myc expressions were observed in polygonal cells, suggesting that polygonal cells have higher proliferation capacity (3). In this study, we showed that there are high levels of Wnt1 and β-catenin expressions in LCH. These findings suggest that LCH is a vascular neoplasia resulting from the increased canonical Wnt1 signaling pathway expression. This increase is observed in both mucosal and cutaneous LCH, revealing that this pathway is jointly used in developing mucosal and cutaneous LCH.

In LCH, we observed increased Wnt1 expression in vascular endothelial cells and tumor stroma. The nuclear and cytoplasmic β-catenin expression in vascular endothelial cells was remarkable. Wnt1 immunoreactivity in tumor stroma suggests that paracrine factors may have influenced this increase. Wnt ligands can be secreted from the tumor microenvironment (16). In addition, some growth factors secreted from stromal and inflammatory cells can activate the expression of Wnt through a secondary route. For example, it has been shown that there is an increase in the hepatocyte growth factor receptor in colorectal carcinoma, which further contributes to tumor progression by increasing the expression of β-catenin (16). In addition, the nuclear translocation of β-catenin and the promotion of epithelial-mesenchymal transition are increased by platelet-derived growth factor (PDGF) (17). Studies on inflammatory cells and tumor interaction in tumoral tissues reveal that macrophages also play a crucial role in tumor progression and angiogenesis (18). Producing Wnt7b, macrophages activate the canonical signaling pathway through paracrine mechanisms in vascular endothelial cells and contribute to tumoral angiogenesis (19). LCH presents a mixed inflammatory

infiltrate, containing lymphocytes, neutrophils, plasma cells, and macrophages. We consider that Wnt ligands secreted from the tumor microenvironment and some growth factors, such as fibroblast growth factor (FGF) and PDGF, may be involved in Wnt signaling upregulation in the pathogenesis of LCH on an inflammatory background.

Epulis gravidarum is a mucosal form of LCH that occurs during pregnancy or oral contraceptive use in women. Female sex hormones have been implicated in the pathogenesis of this condition, and it has been suggested that these hormones cause vascular proliferation by increasing the secretion of basic FGF, vascular endothelial growth factor, and interleukin 1β (20). Katoh reported that Wnt1 was overexpressed in human breast cancer, and β-estradiol upregulated Wnt1 in human breast cancer cell culture (MCF-7 cells) (6). This interaction between estradiol and the Wnt1 signaling pathway may contribute to the increased incidence of LCH during oral contraceptive use or pregnancy.

LCH, especially in the presence of mucosal and finger involvement, may be caused by chronic trauma (21). Mechanotransduction stimulates lymphatic vessel development through activation of the canonical Wnt pathway (22). It is also known that *de novo* vasculogenesis can secondarily develop from endothelial stem cells due to trauma in adults (2). Studies that will reveal the relationship of the Wnt1 signaling pathway with vasculogenesis secondary to trauma and mechanotransduction may illuminate the pathogenesis of LCH caused by trauma.

Myofibroblasts are cells with ultraslow contractility found in inflammatory processes, cancer tissues, and fibrotic tissues. It has been shown that myofibroblasts are also in LCH along with endothelial and pericyte cells (23). They are intensely observed in patients with atypical mucosal LCH and multiple LCHs (24). The importance of the signaling pathway of canonical WNT/ß-catenin has been shown in transforming mesenchymal stem cells into myofibroblasts (25). We think that Wnt1 upregulation is likely to increase the transformation of mesenchymal stem cells into myofibroblasts, which contributes to the fibrotic process in late lesions. Further studies in early and late LCH lesions can demonstrate the relationship between myofibroblasts and the Wnt1 signaling pathway.

Currently, therapeutic approaches that target aberrant canonical Wnt signaling pathway are gaining considerable attention in the field of cancer treatment. Blockade of Wnt1-mediated signaling with the monoclonal anti-Wnt1 antibody has been shown to inhibit survivin and rapidly and significantly induce apoptosis in lung, breast, mesothelioma, and sarcoma cancer cell cultures (26). Similarly, monoclonal antibodies designed to bind Wnt1 and Wnt2 have been shown to suppress tumors in several malignancies, such as colorectal cancer, melanoma, and non-small-cell lung carcinoma (27). Treatment modalities targeting Wnt1 may also be promising in the treatment of LCH.

Study Limitations

The most important limitation of this study is that Wnt1 and β-catenin expression in LCH lesions was evaluated only with the immunohistochemical method. Our second limitation is that we did not screen predisposing factors influential in the emergence of lesions. We consider that the evaluation of genetic mutations associated with the Wnt1/β-catenin signaling pathway and the demonstration of its relationship with predisposing factors will significantly contribute to the elucidation of the pathogenesis of LCH.

Conclusion

This study revealed that the Wnt1/β-catenin signaling pathway in LCH is activated. Further studies on this subject will help reveal the cause of this activation and elucidate the pathogenesis of LCH. Treatment methods targeting Wnt1 may be promising for treating LCH.

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Ethics Committee Approval: The study was approved by the Fırat University Non-Interventional Research Ethics Committee (approval number: 3956, date: 28.09.2021). Because the samples were obtained from the archives of the Fırat University Faculty of Medicine, Department of Pathology, patient consent was not required.

Informed Consent: Because the samples were obtained from the archives of the Fırat University Faculty of Medicine, Department of Pathology, patient consent was not required.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: No conflict of interest was declared by the authors.

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Role of Fungal Species in the Etiology of Nasal Polyposis

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ABSTRACT

Introduction: To investigate fungal species in nasal polyps (NP) by microscopy, fungal culture, fungal DNA isolation, and sequencing.

Methods: Twenty-four patients who applied to our outpatient clinic with complaints of chronic sinusitis and were found to have bilateral NP on clinical examination were included in our study. A control group was formed from 20 patients without NP who underwent septoplasty and endoscopic concha bullosa resection in our clinic. Samples from the participants were subjected to the same microbiological evaluations and the two groups were compared.

Results: The mean age of the patients included in our study was 38.14±14.13 years (range from: 17 to 80). Nine of the participants were female and 35 were male. Direct microscopy and fungal culture positivity rates did not significantly differ between the groups ($p>0.05$). A significant ($p<0.05$) lower rate of microorganisms was detected in tissue cultures obtained from the nasal polyp group. Polymerase chain reaction (PCR) techniques were unable to identify the fungal species in any of the positive fungal cultures. By sequencing, fungal species emerged at similar rates in both groups ($p>0.05$).

Conclusion: We concluded that fungal colonization is not more frequent in patients with NP than in the normal population. We did not observe the superiority of PCR-based sequencing over conventional fungal isolation techniques. However, larger series using molecular methods are needed.

Keywords: Nasal polyps, fungi, sequence analysis

Introduction

Nasal polyps (NP) are benign growths arising from the mucous layer of the nasal cavity and paranasal sinuses due to chronic inflammation. Its prevalence in the general population has been reported as 1-4% (1). They can occur in conditions such as chronic sinusitis, allergies, asthma, and aspirin intolerance. NP can cause symptoms such as nasal congestion, facial pain, nasal discharge, and loss of smell. Treatment can usually be done with methods such as corticosteroid sprays, antihistamines, and surgery (2).

Although the underlying mechanisms in chronic rhinosinusitis with NP (CRSwNP) are not fully defined, treatment modalities that will control the type 2 inflammatory response are being studied (3). Increased exposure to pathogenic microorganisms or allergen defects in the sinonasal epithelial barrier and the state of the individual's immune system play an important role in the pathogenesis of the disease (4). Bacteria play a role in the etiopathogenesis of chronic inflammatory diseases, but the role of fungi is controversial (5). Although the role of fungi in CRS is unknown, their detection has increased significantly in recent years.

In this study, we aimed to detect fungal cues by microscopy, culture, polymerase chain reaction (PCR), and sequencing in patients with NP and to evaluate the importance of fungi in pathogenesis by comparing them with healthy individuals.

Methods

Ethical Approval

The study was approved at the University of Health Sciences Turkey, İstanbul Training and Research Hospital Institutional Review Board (approval number: 64, date: 11.02.2022). All procedures were carried out

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in accordance with the ethical standards specified in the Declaration of Helsinki. An informed consent form was obtained from all patients.

Patients and Study Design

Twenty-four patients who applied to our outpatient clinic with complaints of nasal congestion, nasal discharge, and loss of smell and were found to have bilateral NP on clinical examination were included in our study. The following groups were excluded from the study: a) other causes of nasal obstruction such as septum deviation, inferior turbinate hypertrophy, b) patients with a history of previous nasal surgery, c) patients who have used intranasal or oral corticosteroid therapy for allergic rhinitis in the past 6 months, d) patients who used antibiotics with the diagnosis of chronic sinusitis in the past 6 months, e) patients with a diagnosis of chronic inflammatory disease or drug use, f) immunosuppressed patients.

A control group was formed from 20 patients without NP who underwent septoplasty and endoscopic concha bullosa resection in our clinic. Nasal polyp samples were taken from the study group in the local surgery room, and middle turbinate mucosa samples were taken from the control group by endoscopic concha bullosa resection under general anesthesia. For direct microscopic examination, fungal culture, and fungal PCR studies, using a sterile aseptic technique, two 2-3 mm^3 nasal tissue samples taken from each patient were placed directly into sterile sample containers containing 1 mL of saline and sent to the microbiology laboratory. The first tissue samples were planted for direct microscopic examination, aerobic culture, and fungal culture. Fungal PCR and DNA sequencing tests were performed on the second tissue samples.

Direct Microscopic Examination

Tissue biopsy specimens, of which direct preparations were prepared by suspending with 10% potassium hydroxide solution, were kept for 20 min and then examined with a light microscope for the presence of fungal hyphae, spores, and yeasts with 10X and 40X objectives (Figure 1).

Fungal and Aerobic Culture

Fungal culture: Biopsy samples were inoculated on thioglycolate broth and two separate sabouraud dextrose agar (SDA) (BioMérieux, Lyon, France) media under sterile conditions for fungal isolation. One inoculated SDA was incubated at 26-30 °C and the other at 35-37 °C conditions for 21 days to monitor fungal growth. Gram-stained preparations were prepared from suspicious colonies. Fungal colonies containing yeast or hyphae were evaluated for colony and conidial morphologies and defined at the species level.

Aerobic culture: After incubation of the biopsy samples inoculated in thioglycolate broth for 18-24 hours at 37 °C, passages were taken into 5% sheep blood agar, chocolate agar, and EMB agar media and incubated for 48 h in terms of bacterial growth. Colony morphology and Gram staining characteristics of the cultures with growth were determined and pre-identification was made at the species level using conventional methods. Then, species-level identification was performed using Vitek 2 GN and GP cards in the VITEK® 2.0 Compact (BioMérieux, Lyon, France) automatic ID/AST system for bacterial typing.

Fungal DNA Isolation, PCR and Sequencing

Biopsy samples stored at -80 °C were thawed and taken into a sterile Petri dish and cut into small pieces with the help of a scalpel. 500 µL of Buffer ATL (Qiagene) and proteinase K (50 ng/mL) were added and incubated at 56 °C for 1 h. After homogenization, nucleic acid isolation was performed with the ZymoBiomics® DNA Miniprep Kit (Zymo, CA, U.S.), designed to purify DNA from various sample inputs for microbiome or metagenome analysis according to the manufacturer's instructions (https://doi.org/10.1371/journal.pone.0241732).

The same procedure was used for strains isolated from the culture. Following nucleic acid isolation, fungal DNA was investigated by real-time PCR. The D1-D2 domain of 28S ribosomal RNA was targeted with NL1 (5´-GCA TAT CAA TAA GCG GAG GAA AAG-3´) and NL4 (5´-GGT CCG TGT TTC AAG ACG G-3´) (Figure 2). Positive bands were purified with NucleoSpin® Gel and the PCR Cleanup procedure (Machery-Nagel,

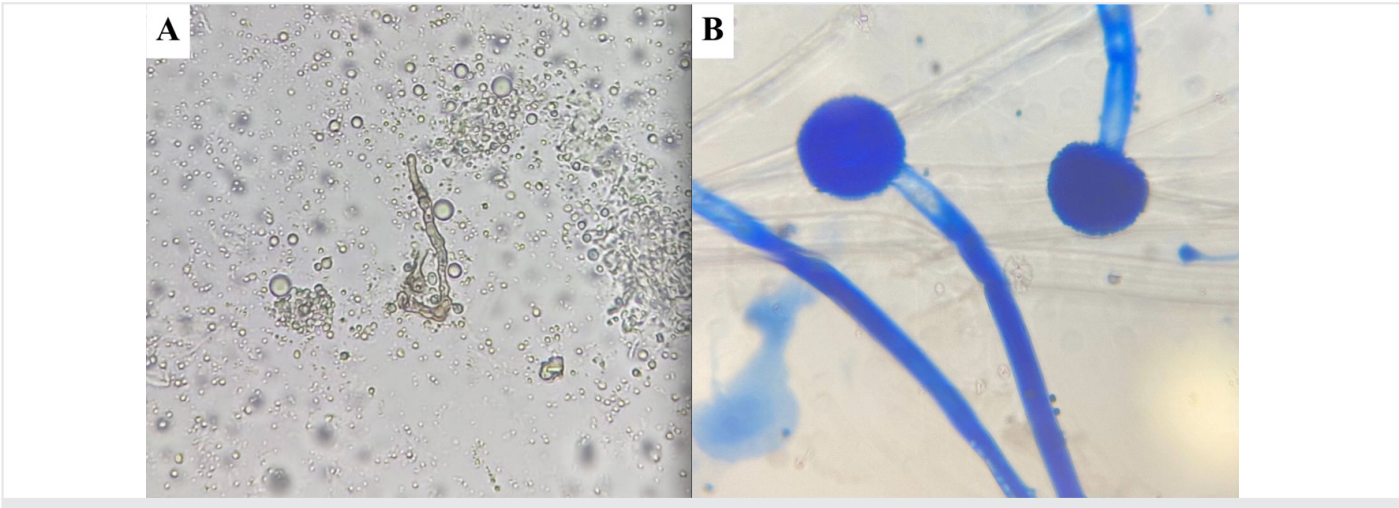

Figure 1. (A) Fungal hyphae visualized in tissue biopsy specimens suspended in 15% potassium hydroxide by direct microscopy. (B) Aspergillus spp. visualized in tissue biopsy specimens suspended in lactophenol cotton blue by direct microscopy

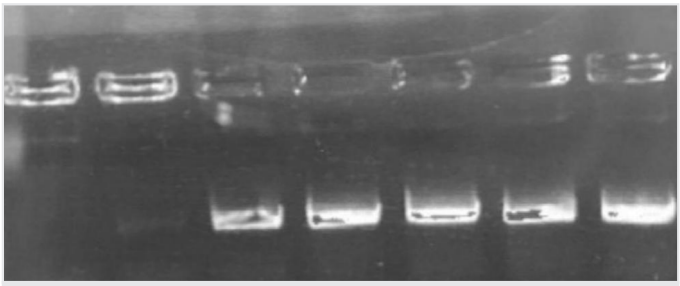

Figure 2. 28s rRNA region of approximately 600 bp amplified with NL1-4 primers (1,2 negative controls, 3 positive control and 4,5,6,7 patient samples)

Cologne, Germany). Bidirectional sequence analysis was performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Massachusetts, U.S.) (http://genomedicine.com/content/5/7/63). Baseline search was performed with sequencing analysis software and consensus sequences were edited with SeqMan software. (Dnastar, Inc., Winconsin, U.S.) For typing of the strains, the most overlapping types were determined by comparison with the sequences defined by blast search.

Statistical Analysis

The IBM SPSS 28.0 package program (SPSS Inc.; Chicago, IL, USA) was used in the analysis. Mean and standard deviation values were used in descriptive statistics of the data. The sample t-test was used in the analysis of independent quantitative data, and the chi-square test was used in the analysis of independent qualitative data. A P-value of <0.05 was considered statistically significant.

Results

The mean age of the patients was 38.14±14.13 years (range from: 17 to 80). Given the possible effect of gender on the findings, there were similar numbers of women in both groups that allow for a fair/valid comparison. Nine of the participants were female and 35 were male.

The age of the patients in the polyp group was significantly higher ($p<0.05$). Gender distribution was similar between the groups ($p>0.05$). The direct microscopy fungus positivity rate did not significantly differ between the groups ($p>0.05$). A significant ($p<0.05$) lower rate of microorganisms was detected in tissue cultures obtained from the nasal polyp group. The fungal culture positivity rate did not significantly differ between the groups ($p>0.05$). PCR techniques were unable to identify fungal species in any of the positive fungal cultures, and by sequencing, fungal species emerged at similar rates in both groups (Table 1). In summary, parallel results were obtained using microscopy, fungal culture, and sequencing.

Discussion

CRS is defined in the European Position Paper on Rhinosinusitis and NP 2012 guidelines as inflammation of the nasal cavity lasting more than 12 weeks with at least two symptoms of nasal congestion, rhinorrhea, facial pain, and hyposmia (6). The phenotype is determined by nasal endoscopy with (CRSwNP) or without polyps (CRSsNP) and the diagnosis is confirmed by paranasal sinus CT. NP are expected to be seen bilaterally in tomography, and when unilateral polyps are seen in adults, care should be taken in terms of malignancy (4).

The pathophysiological process progressing from chronic sinonasal inflammation to nasal polyposis is still not fully defined. It is thought that problems in the sinonasal epithelial barrier may result in prolonged exposure to inhaled pathogens or antigens, thereby increasing chronic inflammation. In CRSwNP, the resistance of sinonasal epithelium is weak and the barrier is defective, but the reason for this is still unclear (7).

It is well known that the upper respiratory tract or paranasal sinuses of patients with NP are often chronically colonized with fungi and bacteria (8). In 2009, the International Society of Human and Animal Mycology classified fungal rhinosinusitis as invasive and non-invasive subtypes, and it was emphasized that invasive types occur in immunosuppressed patients (9). In immunocompetent individuals, non-invasive fungal rhinosinusitis phenotypes present as local fungal colonization, fungus ball, and allergic fungal rhinosinusitis (AFRS). Patients with CRSwNP have predominantly eosinophilic mucin without the fungal invasion. As defined by the Bent-Kuhn criteria, patients with AFRS have a much more intense nasal discharge than patients with NP (10). In addition, Lund-Mackay scores calculated from paranasal sinus CT examination are higher and sinus opacification is more common in patients with AFRS (11).

In 1999, Ponikau et al. (12) in their article investigating the incidence of AFRS found 96% (202 of 210) positivity in fungal culture made from nasal secretions in patients with CRS. With these data presented, they concluded that almost all patients with CRS with or without polyps had positive AFRS diagnostic criteria. In a similar study, Lebowitz et al. (13) isolated fungi in 56% of the samples taken from patients who had undergone endoscopic sinus surgery for CRS. However, negative clinical experiences recently have called into question the role of fungi in the etiology of CRSwNP. In our study, although fungal culture positivity was more common in patients with NP than in those without CRS, no significant difference was found.

PCR-based technologies including sequencing were introduced in the mid-1990s and are considered more effective than conventional methods in detecting fungi (14). While investigating the presence of fungus in the sinus mucosa, Rao et al. (15) in a study that compared PCR with conventional methods detected fungal DNA in 6.5% of patients in whom fungus could not be detected by conventional methods. In our study, no fungal species could be detected in positive fungal cultures and the control group using PCR techniques. This may be due to the absence of fungal DNA in the control samples or the presence of fungal DNA in our sample below the DNA detection limit of the PCR kit.

Today, sequencing is a frequently used alternative for the detection and identification of fungi (16). The sequencing kit used in this study targeted 28S rRNA. Targeting the 18s ribosomal RNA gene in sequencing is not specific to fungi and may also indicate the eukaryotic contamination. Therefore, the internally replicated spacer region, which is more specific to fungi, is used as an alternative target, and this method is the most likely to identify fungi (17). Zhao et al. (17) in their study of 64 patients with CRS concluded that this method cannot be a universal determinant of sinus disease pathogenesis in all CRS patients.

Table 1. Statistical comparison of the groups by each technique individually

Mean ± SD/(n, %)		Control group	Nasal polyp group	p	
		Mean ± SD/(n, %)			
Age		31.55±9.145	43.63±15.345	0.004	t
Gender	Female	4 (20.0%)	5 (20.8%)	0.946	x^2
	Male	16 (80.0%)	19 (79.2%)		
Microscopy					
Negative		19 (95.0%)	21 (87.5%)	0.614	x^2
Positive		1 (5.0%)	3 (12.5%)		
Gram-positive cocci		1 (5.0%)	1 (4.2%)		
Fungal hyphae		0 (0.0%)	2 (8.3%)		
Tissue culture					
Negative		2 (10.0%)	13 (54.2%)	**0.002**	x^2
Positive		18 (90.0%)	11 (45.8%)		
Aspergillus spp.		0 (0.0%)	1 (4.2%)		
Citrobacter koserii		1 (5.0%)	0 (0.0%)		
Diphtheroid bacilli		4 (20.0%)	0 (0.0%)		
Escherichia coli		0 (0.0%)	1 (4.2%)		
Klebsiella pneumoniae		0 (0.0%)	1 (4.2%)		
CoNS		6 (30.0%)	2 (8.3%)		
Leuconostoc mesenteroides		1 (5.0%)	0 (0.0%)		
Alpha hemolytic streptococci		0(0.0%)	1 (4.2%)		
Penicillium spp.		1 (5.0%)	2 (8.3%)		
Staphylococcus aureus		1 (5.0%)	0 (0.0%)		
Staphylococcus aureus MS		1 (5.0%)	2 (8.3%)		
Staphylococcus epidermidis		1 (5.0%)	0 (0.0%)		
Staphylococcus hominis MR		0 (0.0%)	1 (4.2%)		
Staphylococcus hominis MS		1 (5.0%)	0 (0.0%)		
Staphylococcus lugdunensis		1 (5.0%)	0 (0.0%)		
Fungal culture					
Negative		19 (95.0%)	21 (87.5%)	0.614	x^2
Positive		1 (5.0%)	3 (12.5%)		
Aspergillus spp.		0 (0.0%)	1 (4.2%)		
Penicillium spp.		1 (5.0%)	2 (8.3%)		
PCR	(-)	20 (100.0%)	24 (100.0%)	1.000	x^2
	(+)	0 (0.0%)	0 (0.0%)		
Sequencing					
Negative		19 (95.0%)	21 (87.5%)	0.614	x^2
Positive		1 (5.0%)	3 (12.5%)		
Aspergillus sydovii		0 (0.0%)	1 (4.2%)		
Penicillium griseoroseum		1 (5.0%)	2 (8.3%)		

t: T-test, x^2: Chi-square test, CoNS: Coagulase-negative staphylococci, MS: Methicillin-sensitive, MR: Methicillin-resistant

In a study published by Aydil et al. (18) in 2007, they evaluated microscopy and PCR as more sensitive than fungal culture. Unlike this study, we obtained similar results in microscopy, fungal culture, and sequencing. In a 2012 study by Montone et al. (19) on 400 patients with fungal rhinosinusitis, Aspergillus sp. was the most frequently isolated fungus in culture. In a similar study by Eyigor et al. (20), sequence analysis showed that the amplicons were homologous to Cladosporium herbarum and Aspergillus amstelodami. We also observed a only Aspergillus and Penicillium species in our study.

Study Limitations

The main limitation of the study is that it was conducted with a limited sample. Additionally, the long-term postoperative follow-up of patients may help evaluate the relationship between fungal colonization and nasal polyp recurrence.

Conclusion

The importance of fungal etiology in chronic sinusitis patients with NP is still a controversial issue. Microorganism rates isolated in fungal cultures are at similar levels in patients with nasal polyp rhinosinusitis and healthy individuals. This suggests that fungal dysbiosis may not be the only pathogenetic determinant of sinus inflammatory disease. In the findings we obtained in our study, we did not observe the superiority of the PCR-based sequencing technique to fungal culture. However, large case series are still needed.

Ethics Committee Approval: The study was approved at the University of Health Sciences Turkey, İstanbul Training and Research Hospital Institutional Review Board (approval number: 64, date: 11.02.2022).

Informed Consent: An informed consent form was obtained from all patients.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: No conflicts of interest was declared by the authors.

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Evaluation of Thyroid Functions and Its Relationship with Disease Status and Mortality in Hospitalized Patients with COVID-19

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ABSTRACT

Introduction: Hospitalized coronavirus disease-2019 (COVID-19) individuals were studied in terms of their thyroid functioning with respect to their disease severity and mortality rate.

Methods: The thyroid function tests of 781 in patients with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pneumonia outside the intensive care units were examined as part of this retrospective investigation, which was conducted in a single center. Data from the patients were categorized as deceased or discharged. Based on their diagnostic categories, the patients were grouped according to their thyroid stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3) values. TSH, fT4, and fT3 levels were assessed within 24 h after admission.

Results: Of the 781 patients who had COVID-19 of, 726 were discharged and 55 died. When compared to the discharged group, deceased patients exhibited lower than normal TSH and fT3 levels ($p<0.001$; for both). Notwithstanding, there was no significant difference between deceased and discharged patients regarding fT4 values. 115 (14.7%) patients had thyroid dysfunction (16 patients had elevated TSH, 99 had TSH levels below the reference value) and 154 (19.7%) patients had non-thyroidal illness (NTI). The individual effect of thyroid function tests on patient death was investigated using the log rank test, and fT3 levels were found to be significant for predicting mortality.

Conclusion: Our findings imply that thyroid function tests, especially in severe patients, may have prognostic significance. Lower fT3 and TSH levels may be associated with systemic inflammation, which could be a prognostic value associated with the disease state and mortality rate. fT3 was shown to be an independent risk factor for death. As a result, approximately 15% of the patients were observed to have thyroid dysfunction and 19.7% were NTI, which were all linked to severe disease status.

Keywords: COVID-19 pneumonia, thyroid function tests, mortality

Introduction

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) epidemic, also named the "coronavirus disease-2019 (COVID-19) pandemic," has been linked to an elevated fatality rate throughout the world. Previously, SARS-CoV-1 brought about a SARS in late 2002 with a mortality rate of 9.6% (1). Although SARS-CoV-2 infection has been notified to have a relatively lower fatality rate of around 1-3% (2), it is believed to directly affect numerous endocrine glands, including the thyroid. Examination of changes in thyroid structure and function has been reported only in a few studies that rely on clinical assessments of blood samples from SARS patients. In postmortem studies, the thyroid glands of five SARS-CoV-1-effected patients were observed to show serious damage to their follicular epithelium and parafollicular cells. The follicular structure was entirely affected in the form of follicular distortion and collapse (3). In other postmortem studies, the existence of the precursors of the virus in follicular cells of the thyroid and pituitary gland has been demonstrated. Staining results indicated a decrease in the thyroid stimulating hormone (TSH) in the anterior pituitary gland (4,5), although a case series of three COVID-19 patients who received thyroid biopsy did not show any pathological thyroid illness (6).

SARS causes central hypothyroidism in survivors. Among 61 patients who recovered from SARS-CoV-1 with no previous endocrine disease, four (6.6%) had primary hypothyroidism diagnosis (7).

SARS-CoV-2 infects tissues in humans via entry into cells using the angiotensin-converting enzyme 2 (ACE2) receptor, sharing evolutionary similarities with SARS-CoV-1 (8,9). As a result, SARS-CoV-2 entrance into thyroid cells may be caused by the highly expressed ACE2 receptor in the thyroid tissue (10).

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In COVID-19, both the pituitary and hypothalamic tissues indicate ACE2, which renders them possible viral targets (11). Nevertheless, these mechanisms are not yet clarified and the presence of a similar involvement due to SARS-CoV-2 impact either directly or indirectly on the thyroid gland is still unknown (12).

Knowledge regarding how SARS-CoV-2 infection affects the thyroid is fairly limited. Currently, there are few published case reports and studies, especially on thyrotoxicosis due to thyroiditis and non-thyroidal illness (NTI) associated with COVID-19 (13-15).

NTI is a physiological adaptation and/or a pathological response to an acute disease, exhibiting a reduced level of serum T3 and/or T4, although no increase in TSH secretion is observed. Even though its underlying mechanism has not yet been resolved, NTI seems to result from an interaction of physiological adaptation and pathological response to acute illness (16).

In our retrospective research, we investigated thyroid functions and their relationship with mortality in hospitalized COVID-19 patients and evaluated their potential prognostic significance.

Methods

We adopted a cross-sectional and retrospective design in our single-center study. Real-time polymerase chain reaction testing was performed on 1,509 patients who were later diagnosed with COVID-19 and were hospitalized due to COVID-19 pneumonia. These patients who were hospitalized between September 01, 2020 and December 31, 2020 were screened through the database of our hospital, level-3 pandemic, in İstanbul. Patients with known thyroid disease, those who were taking thyroid hormones or antithyroid medications, and those who received any previous head and neck area surgery were excluded from the study. Additionally, individuals with hematological malignancies, advanced carcinoma, rheumatic immune disease, endocrinological disease, organ transplantation, chronic infection disease (such as human immunodeficiency virus, hepatitis C virus and hepatitis B virus), end-stage renal failure and chronic dialysis patients, pregnancy or breastfeeding, and those without clinical features and laboratory values were not included in the study. Patients receiving glucocorticoids and amiodarone were also excluded from the study. All patients were older than 18 years and were not admitted to the intensive care unit (ICU). Seven hundred and eighty-one COVID-19 hospitalized patients enrolled in the study whose demographic data and comorbidity were documented. Upon admission to the hospital, all the patients underwent examinations related to COVID-19, which consisted of recording the respiration rate and initial oxygen saturation by pulse oximetry (SpO_2) to assess the level of oxygen requirement and the radiological status based on spiral computerized tomography (CT). The pulmonary involvement on the chest CT scan was identified as mild, moderate, and severe (17). Data were categorized as deceased and discharged patients. Patients were classified as having moderate and severe illness (18). Within 24 h of admission, blood samples from patients were taken from a peripheral vein. A Roche Cobas C 601 (Roche Diagnostic Limited, Switzerland) device was used to measure thyroid function tests. Individuals were classified into diagnostic categories with respect to their fT4, fT3 and TSH values. In our laboratory, the reference ranges of fT3, fT4 and TSH were 2.5-3.9 pg/mL, 0.61-1.12 ng/dL and 0.34-5.6 µIU/mL, respectively. Patients with NTI were characterized by low fT3 (<2.5 pg/mL) with normal/low TSH and fT4.

The Medical Research Ethics Committee of the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital accepted the research (approval number: 2021/127, date: 15.03.2021). We are dedicated to uphold the Declaration of Helsinki and safeguarding patients' privacy. All the cases included in the study were managed in accordance with the COVID-19 treatment plan of the Turkish Health Ministry (19). All patients provided written informed consent.

Statistical Analysis

Mean and standard deviations were used to express descriptive statistics. Deviation from normality was determined using percentage and median distribution. Normally distributed continuous variables and the categorical data were examined using the chi-square test and Student's t-test. Continuous variables having an abnormal distributions were assessed by Mann-Whitney U test. Statistical significance was validated for a $p<0.05$. Commercially available SPSS software v.21 Statistical Package for the Social Sciences Inc. (Chicago, IL, USA) was used in all statistical analyzes.

The best parameters for predicting mortality from thyroid function tests were obtained from the receiver operating characteristic curves and were later used in the Cox regression model. The possible factors determined by multivariate analyzes with backward selection were fed into the Cox regression model for identifying independent predictors of mortality. The univariate effects of age, gender, arterial hypertension, diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease, C-reactive protein (CRP), procalcitonin, albumin, fibrinogen, D-dimer, troponin I and free T3 (fT3) on death of patients were examined by the log rank test. The residual analysis (Schoenfeld and Martingale) was used to evaluate the proportional hazard assumption and model fit.

Results

Among 781 individuals who had COVID-19 of enrolled in this research, 726 were discharged and 55 died. When the demographic data and coexisting conditions of the patients were investigated, no difference in sex was found between the deceased and survivors. The deceased individuals were older than the discharged group. Hypertension, prior coronary artery disease, prior stroke, heart failure, and atrial fibrillation were significantly more frequent ($p<0.001$, $p=0.002$, $p=0.009$, $p=0.001$, $p=0.003$, respectively) among deceased patients than the discharged. SpO_2 values initially and with supplemental oxygen were significantly lower in deceased patients than in the discharged. Respiratory rate and need for oxygen supplementation were significantly higher in deceased patients. Lymphopenia and platelet counts were significantly lower, whereas neutrophil counts were significantly higher in the deceased group than in the discharged. Urea, creatinine, and aspartate transaminase levels were also significantly higher in deceased patients than in discharged patients. Additionally, deceased patients were associated with stronger inflammatory responses and exhibited poor prognostic laboratory test results, such as CRP, lactate dehydrogenase, D-dimer, fibrinogen, ferritin, and troponin I. Although calcium and albumin were significantly lower in the severe individuals, there was no difference in procalcitonin between groups. Regarding their CT results, the deceased group showed severe involvement compared with the discharged group. Among 781 patients with COVID-19 pneumonia, 53 (96.4%) were severe in deceased patients and 368 (50.7%) were severe in discharged patients (Table 1). TSH and fT3 values were significantly lower

Table 1. Evaluation of demographical and clinical data, laboratory, CT results, and disease status

	Deceased (n=55)	Discharged (n=726)	p
Age (years)	71 (44-96); 71.18±10.88	60 (20-98); 59.89±14.97	<0.001
Gender (F/M) (%)	22/33; (40/60%)	316/410; (43.5/56.5%)	NS
Arterial hypertension on treatment	39 (70.9%)	327 (45%)	<0.001
Diabetes mellitus on treatment	25 (45.5%)	252 (34.7%)	NS
Dyslipidemia on treatment	5 (9%)	27 (3.7%)	NS
Prior coronary artery disease	16 (29%)	94 (12.9%)	0.002
Heart failure	9 (16.4%)	32 (4.4%)	0.001
Chronic atrial fibrillation	7 (12.7%)	23 (3.2%)	0.003
Prior stroke	6 (10.9%)	21 (2.9%)	0.009
COPD¶¶	2 (3.6%)	28 (3.9%)	NS
Asthma bronchial	4 (7.3%)	74 (10.2%)	NS
Baseline SpO_2 (%)	89 (87-93)	95 (94-99)	<0.001
O_2 support (L/per min)	15.67±9.9	3.32±5.4	<0.001
SpO_2¶	92.51±2.23	94.53±1.97	<0.001
The respiratory rate (per minute)	30.89±4.89	20.05±4.51	<0.001
Body temperature (°C)	37.03±0.64	37.02±0.69	NS
Systolic blood pressure (mmHg)	126.51±23.51	127.68±18.84	NS
Diastolic blood pressure (mmHg)	67.13±12.07	71.48±10.3	0.01
Heart rate (per minute)	85.95±16.39	84±14.14	NS
Neutrophil count	7.3±4.54	5.13±2.57	<0.001
Lymphocyte count	0.88±0.71	1.23±0.57	<0.001
Platelet count	214.15±103.58	249.59±107.01	0.01
Htc (%)	36.82±5.08	37.43±4.61	NS
Glucose (mg/dL)	165.92±74.42	150.94±68.2	NS
Urea (mg/dL)	66.74±38.61	38.88±25.27	<0.001
Creatinine (mg/dL)	1.37±1.45	0.95±0.82	0.001
AST (U/L)	51.33±26.61	42.19±29.22	0.02
ALT (IU/L)	39.05±28.57	41.87±39.28	NS
LDH (U/L)	497.80±232.85	327.85±161.75	<0.001
Sodium (mmol/L)	136.38±5.44	137.13±3.76	NS
Potassium(mmol/L)	4.23±0.6	4.16±0.5	NS
Magnesium (mg/dL)	2.01±0.32	2.01±0.28	NS
Calcium (mg/dL)	8.34±0.64	8.79±0.64	<0.001
C-reactive protein (mg/L)	152.01±76.68	96.86±73.46	<0.001
Procalcitonin (ng/mL)	1.04±3.31	1.03±10.70	NS
Albumin (g/dL)	32.42±5.27	36.28±5.32	<0.001
Ferritin (mcg/L)	816.03±754.84	431.66±472.61	<0.001
Troponin I (ng/mL)	67.22±245.2	14.62±58.08	<0.001
D-dimer (mcg FEU/mL)	1.09±1.11	0.77±1.17	0.04
Fibrinogen (mg/dL)	555.45±156.37	490.32±117.62	<0.001
Free T3 (pg/mL)	2.24±0.50	2.80±0.63	<0.001
Free T4 (ng/dL)	1.18±0.44	1.13±0.31	NS
TSH (µIU/mL)	1.18±2.15	1.65±2.64	<0.001
Thorax CT			<0.001
Mild involvement	3 (5.5%)	189 (26%)	
Moderate involvement	13 (23.6%)	339 (46.7%)	
Severe involvement	39 (70.9%)	198 (27.3%)	
Disease status			<0.001
Moderate	2 (3.6%)	358 (49.3%)	
Severe	53 (96.4%)	368 (50.7%)	
The duration of hospitalization (day)	13.85±8.89	11.40±6.64	0.01

¶: Under oxygen support; median, ¶¶: COPD: Chronic obstructive pulmonary disease, CT: Computed tomography, F: Female, M: Male, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase

in deceased patients than in discharged patients within the reference range. No significant difference in free T4 (fT4) value was found between the groups. TSH and fT3 distributions are shown in Figure 1, 2.

The univariate regression analysis demonstrated that gender, age, prior coronary artery disease, arterial hypertension diabetes mellitus, chronic obstructive pulmonary disease, albumin, CRP, fibrinogen, procalcitonin, troponin I, d-dimer, and fT3 were significantly associated with mortality. Cox multivariate regression analysis showed that fT3 was an independent risk factor for mortality (Table 2).

Among 781 patients with COVID-19 pneumonia, 154 (19.7%) were diagnosed with NTI. Only a small number of patients 16 (2%) had TSH levels above the reference value, 13 (1.6%) patients were subclinical with high TSH and normal fT4, 3 (0.4%) patients showed overt hypothyroidism with low fT4 and high TSH. In addition to that there were 99 (12.7%) patients whose TSH levels were below the reference value, 25 (3.2%) patients were subclinical with a low TSH and normal fT4, and 74 (9.5%) showed overt thyrotoxicosis with low TSH and high fT4.

Patients with elevated TSH were observed to be younger and had better clinical status with a lower mortality rate compared with the suppressed TSH and NTI patients. Patients with suppressed TSH levels had an average age of 65 years and had severe clinical status requiring ICU support with a mortality rate of 17.2%. Similar to those in the suppressed TSH patients, average age, clinical status, and mortality rate were higher in NTI patients. Among the 154 (22 deceased/132 discharged) NTI patients, 67 (43.5%) were moderate and 87 (56.5%) were severe, with a mortality rate of 14.3% (Table 3).

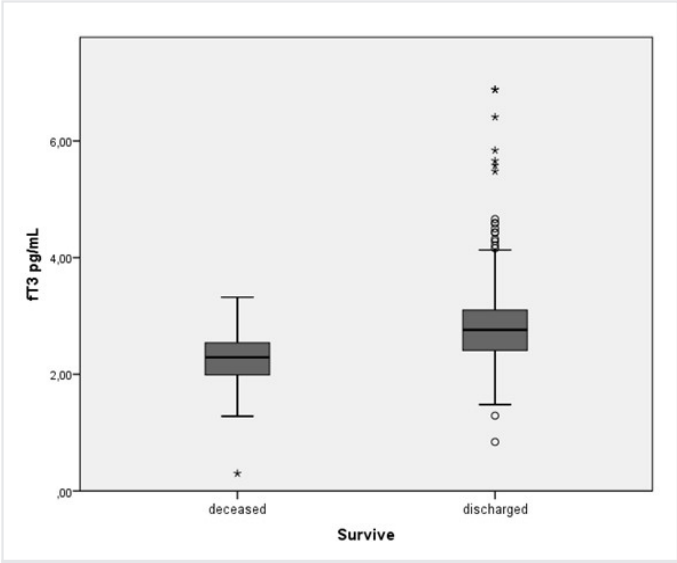

Figure 1. Deceased patients show significantly lower fT3 levels than the discharged (2.24±0.50 vs. 2.80±0.63 pg mL, p=0.001)

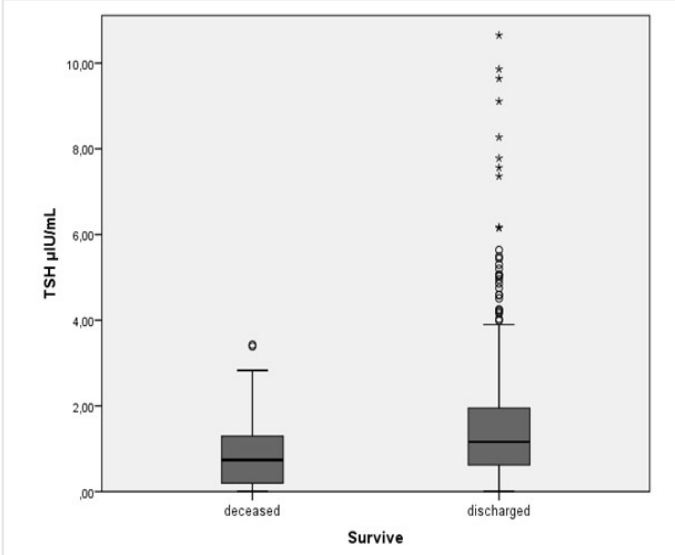

Figure 2. Deceased patients show significantly lower TSH levels than the discharged (1.18±2.15 vs. 1.65±2.64 µIU mL, p=0.001)
TSH: Thyroid stimulating hormone

Table 2. Univariate log-rank and multivariate Cox regression analysis of the risk factors associated with mortality in patients with COVID-19

Variable	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Age	1.044	1.022-1.066	**<0.001**	1.027	0.999-1.057	0.058
Gender	0.911	0.529-1.567	0.736			
Arterial hypertension	2.043	1.139-3.667	**0.017**	1.159	0.501-2.683	0.730
Diabetes mellitus	1.283	0.753-2.186	0.360			
Prior coronary artery disease	1.897	1.055-3.412	**0.032**	1.349	0.618-2.944	0.451
Chronic obstructive pulmonary disease	0.797	0.193-3.289	0.754			
C-reactive protein	1.003	1.001-1.006	**0.008**	0.998	0.993-1.002	0.341
Procalcitonin	1.001	0.976-1.027	0.911			
Albumin	0.924	0.874-0.977	**0.006**	0.936	0.874-1.001	0.055
Troponin I	1.001	0.999-1.002	0.260			
D-dimer	0.962	0.814-1.136	0.645			
Fibrinogen	1.002	1.000-1.004	**0.015**	1.002	0.999-1.004	0.135
Free T3	0.212	0.115-0.390	**<0.001**	0.397	0.201-0.793	**0.008**

COVID-19: Coronavirus disease-2019, HR: Hazard ratio, CI: Confidence interval

Table 3. Thyroid function abnormalities among all patients

	Elevated TSH, µIU/mL >5.6, (n=16)	Supressed TSH, µIU/mL <0,374, (n=99)	NTI TSH, µIU/mL <5.6 and (n=154)
Subclinical/overt	13 (1.6%)/3 (0.4%)	25 (3.2%)/74 (9.5%)	154 (19.7%)
FT4, ng/dL (0.61-1.12)			(FT4 <1.12)
Age	60.5±4.02 (20-85)	64.55±14.73 (37-96)	66.27±15.4 (27-103)
Sex			
Female	10 (62.5%)	41 (41.4%)	83 (53.9%)
Male	6 (37.5%)	58 (58.6%)	71 (46.1%)
The duration of hospitalization (day)	12.69±1.08	14.42±9.72	12.28±7.65
Disease severity			
Moderate	8 (50%)	23 (23.2%)	67 (43.5%)
Severe	8 (50%)	76 (76.8%)	87 (56.5%)
Mortality	1 (6.3%)	17 (17.2%)	22 (14.3%)

NTI: Nonthyroidal illness syndrome, TSH: Thyroid stimulating hormone

Among all deceased patients, one had elevated TSH and 17 had suppressed TSH. Eleven of the suppressed TSH patients showed overt thyrotoxicosis. Moreover, among the deceased patients 22 suffered from NTI.

Discussion

There are few studies in the literature focusing on the evaluation of thyroid function or thyroid pathology on COVID-19. In addition to their scarcity, the results reported in these studies show a certain sense of ambiguity, which may be attributed to thyrotoxicosis or NTI. Evidence shows that there are various effects of SARS-CoV-2 on the thyroid system (11,15).

In our study, we examined the acute impacts of COVID-19 on thyroid function in the largest cohort of patients. Individuals with suppressed TSH levels had severe clinical status and high mortality. More than half of the overt thyrotoxicosis patients having lowered TSH levels and ICU support had severe clinical status with a mortality rate of 17.2%. The mortality rate of NTI patients was 14.3%.

Identification of the thyroid state is important because thyrotoxicosis is believed to arise from several different conditions. Also, tests such as thyroid autoantibodies, ultrasonography, or scintigraphy could not be applied to our patients, most of whom were in severe status due to pandemic conditions.

Several studies reported lower levels of TSH and fT3 within the reference range in patients with COVID-19 (14,15,20-23). Only Chen et al. (20) reported that thyroid function did not predict SARS-CoV-2 infection or progression leading to respiratory failure. Other studies also concluded that thyroid dysfunctions were correlated with COVID-19 severity. They demonstrated the link between thyroid dysfunction and prognosis as they suggested that thyroid dysfunction was associated with a higher mortality and a prolonged hospitalization in individuals with SARS CoV-2 (14,21-24).

Chen et al. (21) in a retrospective study found lower T3 and TSH levels in 18% of the patients, together with a correlation to severe disease. In another study, Lania et al. (13) similarly identified overt thyrotoxicosis in 10.8% of their cohort of 287 individuals with SARS-CoV-2 who were managed without ICU, although they measured thyroid hormones in only 25% of their patients. Similarly, we found lower TSH and fT3 levels in 12.7% of the patients, with a positive correlation to the severity of COVID-19 infection. Similar to our results, a further study by Gao et al. (23) showed that fT3 levels at baseline (but not fT4 or TSH) acted as independent predictors of mortality in their cohort of patients. We observed a decrease in both fT3 and TSH levels, which were positively related to mortality in a larger group of patients. Additionally, fT3 and TSH levels in deceased patients were significantly lower than those in discharged patients.

On the other hand, current observational research by Khoo et al. (25) provided the acute effects of COVID-19 on thyroid function in the largest known cohort of patients so far. Their results showed that a major group of patients were euthyroid, while only a small group was subclinical hypothyroid (5.1%) or overt hypothyroid (0.6%). Eight patients were suspected of secondary hypothyroidism (2.4%). Neither subclinical nor overt thyrotoxicosis was observed in the diagnosis of their patients. The authors determined that in their cohort there was no evidence of a COVID-19-associated overt thyroid dysfunction, but their results were more indicative of a NTI syndrome (25).

Recently, data obtained from case reports and small clinical studies indicate that NTI is characterized by a reduction in T3 levels associated with adverse conditions, which may lead to mortality, especially in severe COVID-19 patients (16,26-28).

We tried to demonstrate the relationship between mortality and thyroid function, which implied its prognostic significance. We also studied the relationship between the duration of hospitalization and death rates in a large study group of COVID-19 individuals with non-mild status and having no prior thyroid disease. As a result, thyroid dysfunction was observed in approximately 15% of the patients and was associated with severe clinical condition. In addition, the fT3 level was suggested to be an independent prognostic indicator of mortality. As shown in some previous studies, these findings indicate an association of COVID-19 and thyroid with destructive or autoimmune mechanisms.

COVID-19 disease may have a direct effect on thyroid function. Low normal FT3 and low normal TSH levels may be associated with systemic inflammation and bearing a prognostic significance associated with disease state and mortality. These findings may also be indicative of NTI, which is a probable reason for the changes in thyroid function. A minor decrease in TSH with normal fT4 levels with low-normal fT3 levels was observed in NTI patients. As against the widely accepted thyrotoxicosis argument, this fact may be attributed to an alteration in thyroid hormone metabolism and/or pituitary responsiveness.

In our study, the relationship between mortality and thyroid function, especially around 15% overt thyroid dysfunction and 20% NTI, and its prognostic significance was demonstrated. We showed that fT3 was an independent risk factor for mortality. Our results show the suggestibility of thyroid function tests to be considered, at least in severe patients, even if not in all cases. On the other hand, further studies are needed to investigate the long-term effects of SARS-CoV-2 on thyroid function.

Study Limitations

The fundamental limitations of our study are its retrospective character and its unbalanced female dominance over male subjects (16/781) having overt or subclinical hypothyroidism. Because tests such as thyroid autoantibodies, ultrasonography or scintigraphy could not be performed due to pandemic conditions, we could not study whether patients had chronic thyroiditis, such as Hashimoto's. Furthermore, severe patients had various comorbidities and were also under a commonly prescribed medication. This might have caused some drug interactions as an effect of displacement of the thyroid hormone from the binding proteins such as furosemide, metformin, and salicylates (29).

Thyroid hormones can also alter dynamically with the advance or resolution of the underlying primary disorder. Finally, the potential role of thyroid hormones in COVID-19 needs to be investigated further, although the cost-effectiveness and diagnostic value of serum TSH measurement in hospitalized patients are still controversial (30).

Conclusion

In our study, the relationship between mortality and thyroid function was investigated in COVID-19 patients. Our results suggest that thyroid function tests, especially in severe patients, may have prognostic significance because fT3 is an independent risk factor for mortality. The presence of suppressed TSH in an average of 10-15% of the patients supports the triggering of possible destructive and autoimmune mechanisms and/or pituitary responsiveness. NTI is an independent factor that may influence the changes in thyroid function leading to a severity in patients with COVID-19 pneumonia. Moreover, the presence of suppressed TSH levels and overt thyrotoxicosis in 17 of 55 patients who died indicate the importance of evaluating thyroid functions as etiologic factors in severely ill patients.

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Results of 78 Patients with Idiopathic Granulomatous Mastitis Who Received Peroral Steroid Therapy: Our Clinical Experience

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ABSTRACT

Introduction: For approximately half a century, synthetic steroids have been the most commonly used medical treatment for idiopathic granulomatous mastitis (IGM). We present the outcomes of patients who experienced relapse after systemic peroral steroid treatment (SPST) and achieved remission.

Methods: This study included a total of 78 patients who were diagnosed with IGM and started on SPST from 2010 to 2020.

Results: The mean age of the patients included in the study was 33.4±6.8 years (minimum-maximum: 19.0-53.0 years) and 94.9% of all patients were premenopausal. After the clinicalopathological diagnosis, while complete remission was achieved in 46.2% of the patients receiving SPST at the first session, 53.8% showed resistance to therapy and/or had a relapse. In the group of patients who experienced relapse, the rate of bilateral disease, abscess drainage, and secondary side effects of SPST were significantly higher ($p<0.05$). Of the patients who showed resistance to treatment and/or experienced relapse, 39.7% (31 of 78) achieved remission after a combination of medical treatments and 14.1% (11 of 78) achieved remission after a combination of medical and surgical treatments.

Conclusion: In patients with clinical-radiological presentation of abscess and/or bilateral disease before treatment, the disease tends to have an aggressive course and the frequency of secondary side effects of SPST is higher. These patients should be informed about the risk of relapse, side effects, and combined treatments, and they should be followed up more closely.

Keywords: Breast, idiopathic granulomatous mastitis, steroid, recurrence, risk factor

Introduction

Idiopathic granulomatous mastitis (IGM) is a rare benign disease of the breast. IGM was first defined by Kessler Wolloch in 1972 (1-4). Although the annual prevalence of IGM in the literature is reported to be 2.4 per 100,000 women aged between 20 and 40 years of age, there are large case series reported worldwide, mostly from Eurasian countries (1,3,5).

IGM is diagnosed by ruling out a specific etiology through clinical and pathological means. In terms of differential diagnosis, it can be confused with breast cancers, as well as pyogenic and specific granulomatous mastitis of the breast [infectious specific granulomatous mastitis (such as breast tuberculosis and bacterial, fungal, and parasitic granulomatous mastitis) and non-infectious specific granulomatous mastitis (such as breast sarcoidosis, Wegener's granulomatosis, giant cell arteritis, foreign body, ductal ectasia, fat necrosis, and sclerosing lymphogranulomatous mastitis)] (1-4,6-8).

There are many hypotheses regarding the pathophysiology of the disease. These are the secretion theory, the autoimmune theory, and the geographic or ethnic hypothesis, respectively. Therefore, although a definitive relationship cannot be established, autoimmune diseases, hyperprolactinemia, hormonal imbalances, oral contraceptive (OCP) use, trauma, local irritants, lactation, parity, type 2 diabetes mellitus, smoking, ductal ectasia, and antipsychotic drug use are suggested as predisposing risk factors in etiopathogenesis (2,3,6-12).

Patients diagnosed with IGM based on clinical and pathological findings can be managed with various treatment options, including the watch-and-wait approach and the use of steroids, methotrexate (MTX), azathioprine, bromocriptine, colchicine, non-steroidal anti-inflammatory drugs (NSAIDs), surgical approaches (partial or total mastectomy), and/or combination therapies (2,3,6-10,13-16). Despite the availability of different treatment methods, steroids are currently the most commonly used treatment method for IGM (13-15). In clinical practice, the most commonly used steroid preparations are prednisone, prednisolone, and methylprednisolone, each with varying levels of potency. According to the literature, the equivalent doses of these medications are 20 mg of hydrocortisone, 5 mg of prednisone, 5 mg of prednisolone, and 4mg of methylprednisolone. The daily doses of prednisone are classified into low dose (<7.5 mg/day), medium dose (7.5-30 mg/day), high dose (30-

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100 mg/day), very high dose (>100 mg/day) and pulse therapy (≥250 mg/day) in clinical practice (17,18).

According to the literature, recurrence rates of the disease vary between 0% and 100%, depending on different clinical manifestations and treatment methods reported in various studies (3,6-10,13,19,20). Our aim is to present the outcomes of patients who experienced relapse after SPST between 2010 and 2020 and achieved remission.

Methods

This study was conducted in line with the ethical standards defined by the Institutional Research Committee and the 1964 Helsinki Declaration. Ethics committee approval for the study was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 116, date: 12.05.2023).

Patient selection: We retrospectively reviewed the data of patients aged 18 years and older (n=84) whose IGM diagnosis was confirmed based on histopathological and microbiological findings and who gave consent to SPST in our clinic between 2010 and 2020. The study included patients (n=78) who received at least one course of SPST and had at least one follow-up visit after completion of treatment. Patients (n=6) who were non-compliant with treatment were excluded from the study.

Diagnosis, treatment, follow-up, and data collection: Before treatment, we reviewed the medical records of the patients to collect information on their coexisting systemic diseases, demographic data, physical examination findings, viral hepatitis, brucellosis (Rose Bengal plaque agglutination test), antinuclear antibodies, anti-ds DNA, hyperprolactinemia, rheumatoid factor, non-specific acute phase reactants, hemogram index parameters (HIP), and basic biochemical tests. Radiological assessments were performed to evaluate sarcoidosis and exposure to *Mycobacterium tuberculosis*, including chest X-rays. Breast ultrasound and/or magnetic resonance imaging (MRI) were used to assess the extent of the disease, and mammography results were reviewed for patients over the age of 40 (1-3,6,11,12,21).

The patients' tissue biopsies (tru-cut or abscess wall); were examined for the presence of non-caseating granulomas, along with lymphocytes, plasma cells, and epithelioid histiocytes that were rarely accompanied by eosinophils in and around the lobules, as well as Langhans giant cells. The presence of microorganisms (by gram staining for bacteria, periodic acid-Schiff staining for fungi, Erlich-Ziehl-Neelsen staining/polymerase chain reaction for tuberculosis) and the absence of a specific etiology were also checked. Additionally, microbial negativity was recorded by culturing and microscopically examining tissue biopsies for fungal, parasitic, and bacterial infections (1-4,6,14).

Patients with acute mastitis symptoms were treated with empirical antibiotic therapy and NSAIDs for 10-15 days until laboratory results were obtained. Those with abscesses received drainage either percutaneously or through an incision. Patients who were confirmed to have IGM after histopathological and microbiological evaluations and gave consent for SPST were started on medium-/high-dose prednisolone (0.5-1 mg/kg/day). The drug dose was gradually reduced after treatment. To prevent the side effects of steroids during treatment, patients were advised to take calcium and vitamin D supplements, proton pump inhibitors, and restrict their intake of salt and carbohydrates (2,3,6,15,17,18). After treatment, the complete disappearance of the mass, inflammation, fistula, and skin lesions in the breast and the absence of disease recurrence were defined as remission. During treatment, the persistence of the disease or its recurrence after treatment was considered a relapse. In addition, the development of IGM in the contralateral breast after treatment was considered a relapse.

The patients who experienced relapse were offered treatment options including a second round of SPST, SPST + MTX, watch and wait, and surgical treatment. Once they gave their consent, the treatment of choice was initiated. MTX treatment was initiated after a short course (3 weeks) of SPST. The patients received a divided dose of 15 mg/week for 6 months. During the treatment, patients were advised to follow a low-carbohydrate and low-salt diet to minimize the side effects of steroids and MTX. Additionally, they were started on calcium, vitamin D, PP, and folic acid to protect against adverse effects. Furthermore, due to the toxic side effects of MTX, hemogram and biochemistry tests were performed every two months to check patients' well-being. In the watch-and-wait method, the patients were followed up using empirical antibiotherapy, NSAIDs, and drainage (percutaneous or incisional) in case of mastitis and/or breast abscess. Our surgical treatment recommendation was either partial or total mastectomy with sound surgical margins based on the extent of the disease (2,3,6-10,13-18).

Study design: The patients' HIP, demographic characteristics, and pre-and post-treatment options were recorded. The examination findings were recorded according to the initial presentation side, and the measurement of lesion sizes was performed based on the largest inflamed tumor after clinical and radiological evaluation. After the first session of SPST, patients were grouped based on those who achieved remission and those who were resistant to treatment and/or experienced relapse and were then compared.

Statistical Analysis

We used descriptive statistics of the mean, standard deviation, median, minimum, maximum, frequency, and ratio. The Kolmogorov-Smirnov test was used to measure the distribution of the variables. The independent sample t-test and the Mann-Whitney U test were employed in the analysis of quantitative independent data. Quantitative independent data were analyzed using the chi-square test, but when the conditions for the chi-square test were not met, we used the Fisher's exact test. The SPSS 28.0 program was used for the analyses.

Results

The mean age of the patients included in the study was 33.4±6.8 years (minimum-maximum: 19.0-53.0 years) and 94.9% of all patients were in premenopause. In patients' medical history, 17.9% reported smoking, 14.1% had a history of OCP use, and 92.3% had at least one pregnancy and lactation history. The prevalence of accompanying chronic diseases was 15.4%, with a distribution of 7.7% autoimmune diseases [Hashimoto's thyroiditis (n=4), rheumatoid arthritis (n=2)], 3.8% hypertension (n=3), and 3.8% other disorders [chronic obstructive pulmonary disease (n=1), migraine (n=1), and anemia (n=1)]. In our study group, 55.1% of the cases had IGM originating from the left breast.

The most common symptom and finding was a breast mass with an average size of 5.9±2.6 cm. The distribution of other symptoms and findings was as follows: 98.7% inflammation, 79.5% abscess, 70.5% fistula, and 16.7% nipple retraction. Physical examination findings were evaluated radiologically using US in 100% of cases, MRI in 69.2%, and MG in 11.5% of patients over the age of 40 (Table 1).

Tissue biopsies for histopathological and microbiological diagnosis were obtained from the abscess wall through a mini-incision in 16.7% of cases

Table 1. Distribution of patients receiving peroral systemic steroid therapy after histopathological and microbiological diagnosis

		Min.-Max.	Median	Mean ± SD -%(n)/%(n)
Age		19.0-53.0	32.5	33.4±6.8
Menopause status	Premenopause/ postmenopause			94.9 (74)/5.1 (4)
History of giving birth and breastfeeding	+/-			92.3 (72)/6.4 (5)
Smoking	+/-			17.9 (14)/80.8 (63)
Oral contraceptive	+/-			14.1 (11)/85.9 (67)
Chronic disease	+/-			15.4 (12)/84.6 (74)
	Autoimmune disease			7.7 (6)
	Hypertension			3.8 (3)
	Other diseases			3.8 (3)
Side	Right/left/bilateral			37.2 (29)/55.1 (43)/7.7 (6)
Mass size (cm)		1.5-15.0	5.9	5.9±2.6
Mass	+/-			100 (100)/0 (0)
İnflammation	+/-			98.7 (77)/1.3 (1)
Fistula	+/-			70.5 (55)/29.5 (23)
Nipple retraction	+/-			16.7 (13)/83.3 (65)
Abscess drainage	+/-			79.5 (62)/20.5 (16)
Tissue diagnosis	Abscess wall/tru-cut			16.7 (13)/83.3 (65)
Radiological imaging	US/MRI/MG			100 (78)/69.2 (54)/11.5 (9)
WBC ($x10^9$/L)		4.0-20.0	8.8	8.9±2.8
Neutrophil ($x10^9$/L)		2.2-18.1	5.8	6.1±2.7
Lymphocyte ($x10^9$/L)		0.8-3.5	2.0	2.1±0.7
Monocyte ($x10^9$/L)		0.04-1.08	0.53	0.55±0.21
Basophil ($x10^9$/L)		0.01-0.4	0.03	0.04±0.07
Platelets ($x10^9$/L)		168.0-595.0	302.5	313.0±80.0
NLR		1.19-17.85	2.79	3.48±2.74
PLR		67.6-461.9	146.9	169.1±75.7
LMR		1.3-21.0	3.8	4.3±2.6
LBR		3.9-314.8	82.2	91.7±55.6
CRP (mg/dL)		0.0-78.9	0.8	4.6±12.3
Sedimentation (mm/h)		7.0-106.0	33.0	41.3±25.2
Prednisolone dosage (mg/day)		20.0-90.0	60.0	55.9±17.8
Initial prednisolone time (month)		0.7-2.0	1.0	1.1±0.3
Prednisolone discontinuation time (month)		0.7-4.3	1.4	1.5±0.8
Total prednisolone treatment time (month)		1.4-5.3	2.5	2.6±0.9
Follow-up time (month)		0.5-124.3	60.6	60.5±29.0
Prednisolone side effects	+/-			60.3 (47)/39.7 (31)
	Edema			42.3 (33)
	Hirsutism			6.4 (5)
	Buffalo humb			1.3 (1)
	Arthralgia			7.7 (6)
	Menstrual irregularity			2.6 (2)
Remission/recurrence				46.2 (36)/53.8 (42)

WBC: White blood cells ($x10^9$/L), NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, LMR: Lymphocyte to monocyte ratio, LBR: Lymphocyte to basophil ratio, CRP (mg/dL): C-reactive protein, US: Ultrasonography, MRI: Magnetic resonance imaging, MG: Mammography, Min.: Minimum, Max.: Maximum, SD: Standard deviation

and by tru-cut biopsy in 83.3% of cases. Seventy-seven patients (98.7%) with symptoms of acute mastitis were started on empiric antibiotic therapy and NSAIDs for 10-15 days until their sterility was confirmed by culture and microbiological tests. Sixty-two patients (79.5%) requiring drainage underwent abscess drainage [percutaneous (n=49), mini-incision (n=13)] (Table 1).

For patients in whom no descriptive etiology was found in the tissue samples obtained, oral prednisolone was started at an average dose of 55.9±17.8 mg/day for 1.1±0.3 months in the first session. SPST was gradually discontinued over an average of 1.5±0.8 months. During the treatment, 60.3% of the patients experienced side effects related to the use of steroids. The distribution of side effects was as follows: 42.3% edema, 7.7% arthralgia, 6.4% hirsutism, 2.6% menstrual irregularity, and 1.3% buffalo hump (Table 1).

After clinical and pathological diagnosis, remission was achieved in 46.2% of patients after the first session of treatment, 83.3% after the second session, and 100% after the third session. Partial mastectomy was performed in 14.1% of patients. After the first session of SPST treatment, the rate of bilateral disease, abscess, and secondary side effects due to steroid treatment were significantly higher ($p<0.05$) in the group (53.8%) who were resistant to treatment and/or had a relapse following treatment. There was no significant difference ($p>0.05$) between the patients regarding non-specific acute phase reactants and HIP. Of the patients who showed resistance to treatment and/or experienced relapse, 39.7% (31 of 78) achieved remission after a combination of medical treatments and 14.1% (11 of 78) achieved remission after a combination of medical and surgical treatments (Table 2-4).

Discussion

Although it has been reported in the literature that IGM can occur in all age groups, including children and the elderly (from 11 to 83 years of age), it most commonly presents with a unilateral or bilateral mass in women of reproductive age. In addition to the mass, inflammation,

Table 2. Comparison of clinical data and blood tests between groups before the first session peroral steroid treatments

		Remission		Recurrence		p
		Median	Mean ± SD -%(n)/%(n)	Median	Mean ± SD -%(n)/%(n)	
Age		32.5	33.0±6.5	32.5	33.7±7.1	0.623[t]
Menopause status	Premenopause/ postmenopause		94.4 (34)/5.6 (2)		95.2 (40)/4.8 (2)	1.000[x2]
Birth history and breastfeeding	+/-		97.2 (35)/2.8 (1)		88.1 (37)/9.5% (4)	0.215[x2]
Smoking	+/-		19.4 (7)/80.6 (29)		16.7 (7)/81.0 (34)	0.788[x2]
Oral contraceptive	+/-		19.4 (7)/80.6 (29)		9.5 (4)/90.5 (38)	0.209[x2]
Chronic disease	+/-		16.7 (6)/83.3 (30)		14.3 (6)/85.7 (36)	0.771[x2]
Side	Right/left/bilateral*		47.2 (17)/52.8 (19)/0 (0)		28.6 (12)/57.1 (24)/14.3 (6)	0.030[x2]
Mass size (cm)		5.8	5.7±2.5	5.9	6.1±2.7	0.588[m]
Mass	+/-		100 (36)/0 (0)		100 (42)/0 (0)	1.000[x2]
İnflammation	+/-		97.2 (35)/2.8 (1)		100 (42)/0 (0)	0.462[x2]
Fistula	+/-		66.7 (24)/33.3 (12)		73.8 (31)/26.2 (11)	0.490[x2]
Nipple retraction	+/-		19.4 (7)/80.6 (29)		14.3 (6)/85.7 (36)	0.542[x2]
Abscess drainage	+/-		61.1 (22)/38.9 (14)		95.2 (40)/4.8 (2)	0.000[x2]
Tissue diagnosis	Abscess wall/tru-cut		11.1 (4)/88.9 (32)		21.4 (9)/78.6 (33)	
Radiological imaging	US/MRI/MG		100 (36)/72.2 (26)/13.9 (5)		100 (42)/66.7 (28)/9.5 (4)	
WBC ($x10^9$/L)		8.2	8.9±2.7	9.1	8.9±2.9	0.819[m]
Neutrophil ($x10^9$/L)		5.3	6.0±2.6	6.3	6.2±2.8	0.772[m]
Lymphocyte ($x10^9$/L)		2.1	2.2±0.7	1.9	2.0±0.6	0.190[t]
Monocyte ($x10^9$/L)		0.53	0.54±0.22	0.53	0.56±0.2	0.612[t]
Basophil ($x10^9$/L)		0.03	0.04±0.07	0.02	0.04±0.07	0.238[m]
Platelets ($x10^9$/L)		319.5	3192±72.4	282.5	308.2±85.9	0.259[m]
NLR		2.7	3.3±3.0	2.8	3.6±2.6	0.383[m]
PLR		146.9	163.4±77.9	150.0	173.4±74.7	0.527[m]
LMR		4.2	4.9±3.4	3.6	3.9±1.7	0.099[m]
LBR		77.3	96.0±69.5	85.0	88.5±42.8	0.694[m]
CRP (mg/dL)		1.0	7.3±17.9	0.8	2.4±3.5	0.805[m]
Sedimentation (mm/h)		27.0	34.3±22.1	39.8	46.3±26.5	0.083[t]

[t]: T-test, m: Mann-Whitney U test, [x2]: Chi-square (Fisher's test), WBC: White blood cells ($x10^9$/L), NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, LMR: Lymphocyte to monocyte ratio, LBR: Lymphocyte to basophil ratio, CRP (mg/dL): C-reactive protein, US: Ultrasonography, MRI: Magnetic resonance imaging, MG: Mammography

abscess, ulceration, fistula, nipple retraction, and erythema nodosum can accompany IGM (1-3,6,8,13,14,19,20). These symptoms can also present in breast cancers, pyogenic, and specific granulomatous mastitis (1-4,6-8). The initial radiological evaluation was performed using breast US. With US, the size of the mass, area of inflammation, abscess, sinus formation, and axillary lymphadenopathy can be evaluated in the breast. Additionally, US is a good guide for biopsy and a useful method for follow-up after percutaneous abscess drainage and treatment. In MG requested for screening malignancies in patients over the age of 40, there may be findings of thickening of the skin, distortion, asymmetry (focal/diffuse), and calcification. When the extent of the disease is not adequately assessed by MG and/or US, MRI can be used. However, although radiological methods are useful in the diagnosis of the disease, they cannot clearly distinguish malignancy. The diagnosis of the disease is made by excluding a descriptive etiology after a histopathological, microbiological, and clinical evaluation (1-4,6-10,13-16,19-21).

Our study is consistent with the literature, as the average age of the patients was 33.4±6.8 years years and 94.9% were premenopausal. The most common physical examination finding was a breast mass, and all patients were evaluated with breast US. Tissue biopsies for histopathological and microbiological examination were obtained using a tru-cut needle in 83.3% and from the abscess wall in 16.7% of the patients.

Although 60 years have passed since its first description, the pathophysiology of the disease remains unclear. While IGM is generally regarded as a self-limiting, slowly resolving, benign inflammatory disease in the literature, the management of recurrent and persistent symptoms is still controversial. There is no consensus on the treatment of IGM, where the aim is to achieve the fastest recovery and the lowest recurrence rate after clinical and pathological diagnosis (2,6,7,13,14,16). Prior to 1980, aggressive surgical treatment methods were dominant. However, during this period, the problem was the risk of recurrence due to inadequate partial mastectomy, and the problem was cosmetic dissatisfaction with extensive partial mastectomy. After 1980, with the introduction of immunosuppressive agents, surgical treatment was replaced by medical treatments, the watch-and-wait method, and/or their combinations. Among medical treatments, steroids are the most commonly used anti-inflammatory and immunosuppressive agents (3,6,7,13-16,21,22). In addition to their immunosuppressive effects, steroids also have effects on the hematopoietic, urinary, cardiovascular, and central nervous systems as well as on endocrinological and calcium, lipid, glucose, and protein metabolism (17,18). As a result of using moderate to high doses of systemic glucocorticoids orally, patients may experience side effects such as edema (weight gain), Cushing syndrome, hirsutism, diabetes, and osteoporosis at rates ranging from 0 to 81.3% (9,11,14,21-23). In our study, 60.3% of patients experienced side effects, with edema being the most common affecting 42.3% of patients.

Table 3. Comparison of the first session peroral steroid treatments and side effects between groups

		Remission		Recurrence		p
		Median	Mean ± SD -%(n)/%(n)	Median	Mean ± SD -%(n)/%(n)	
Prednisolone dosage (mg/day)		50.0	52.8±16.1	60.0	58.6±18.9	0.150[m]
Initial prednisolone time (month)		1.0	1.1±0.3	1.0	1.1±0.4	0.875[m]
Prednisolone discontinuation time (month)		1.4	1.5±0.7	1.3	1.6±0.8	0.417[m]
Total prednisolone treatment time (month)		2.3	2.5±0.9	2.7	2.6±0.9	0.640[m]
Follow-up time (month)		59.7	58.2±32.2	61.5	62.4±26.0	0.523[t]
Prednisolone side effects	+/-		47.2 (17)/52.8 (19)		71.4 (30)/28.6 (12)	0.029[x2]

[t]: T-test, [m]: Mann-Whitney U test, [x2]: Chi-square (Fisher's test).

Table 4. After histopathological and microbiological diagnosis, 1st session, 2nd session, 3rd session treatment distribution

	Treatment		Medical/surgical	Recurrence	Remission	Total remission
			% (n)/% (n)	% (n)	%(n)	% (n)
After clinicalopathological diagnosis	1st	P	100 (78)/0 (0)	53.8 (42)	46.2 (36)	46.2 (36)
	2nd	P/P	93.6 (73)/6.4 (5)	9.2 (8)	7.7 (6)	83.3 (65)
		P/SO		1.3 (1)	6.4 (5)	
		P/W-W		1.3 (1)	17.9 (14)	
		P/P + MTX		3.8 (3)	5.1 (4)	
	3rd	P/P/SO	85.9 (67)/14.1 (11)	0.0 (0)	3.8 (3)	100 (78)
		P/P/W-W		0.0 (0)	5.1 (4)	
		P/P + MTX/SO		0.0 (0)	1.3 (1)	
		P/SO/W-W		0.0 (0)	1.3 (1)	
		P/W-W/SO		0.0 (0)	1.3 (1)	
		P/P + MTX/W-W		0.0 (0)	3.8 (3)	

P: Systemic peroral prednisolone therapy or systemic peroral steroid therapy, MTX: Methotrexate, SO: Surgical operation, W-W: Watch and wait

There is no standard treatment approach for IGM, which is a sterile disease, and antibiotics play no role in managing this condition. However, until a definite diagnosis of the disease is made based on clinical and pathological findings, EA may be recommended for patients with clinical signs of mastitis, and drainage (percutaneous or incisional) may be recommended in the presence of an abscess. In addition, antibiotherapy and drainage may also be effective in case of clinical aggravation secondary to contamination from fistulas and ulcerative lesions or in the management of recurrent abscesses following medical treatment after clinical-pathological diagnosis (2,3,6,7,13,16,19,22). In this study, inflammation was present in 98.7% of patients and abscess drainage [percutaneous (n=49), mini-incision (n=13)] was performed in 79.5% of patients before clinical-pathological diagnosis. Due to the 2-week period needed for clinicalopathologic diagnosis in our clinic, short-term empirical antibiotic therapy and NSAIDs were initiated for 98.7% (n=77) of these patients presenting with acute mastitis symptoms. However, after confirming the sterility status, antibiotic and NSAID treatments were discontinued and SPSTs were rearranged.

The disease may present as a non-complicated inflammatory solitary mass or with complicated mastitis symptoms such as an abscess or fistula accompanying the mass in the breast. In 2021, Toktas et al. (14) reported that local steroid treatment is an effective treatment method in patients with non-complicated IGM. It was reported in the study by Ertürk et al. (24) that local steroid therapy was effective for non-complicated lesions smaller than 3 cm, but less effective for complicated lesions presenting with fistulas (39.5%) and abscesses (63.2%) and with a size of 3 cm or larger. According to the authors, this was due to the inability to provide sufficient steroid dosage in patients with fistulas and the re-formation of abscesses after injection in patients with abscesses (24). According to Velidedeoglu et al. (6), in cases of non-omplicated IGM with non-mass or small lesions (lesion size <2 cm), it was enough to follow up with patients via the watch-and-wait approach or with NSAIDs. In a study conducted by Yaghan et al. (25) in 2019, patients were divided into groups based on their presentations as follows: mass (13.23%), inflamed mass (52.94%), abscess (26.47%), and skin lesions (ulcer, sinus, fistula) (7.35%) in the breast. No recurrence was observed in patients who only had a mass and underwent surgery. However, they observed recurrence in 50% of patients who presented with inflammation, abscess, or skin lesions and received both surgical and medical treatment (25). In the study by Tan et al. (20), before treatment, 100% of patients presented with a mass, 71.6% with inflammation, 54.5% with abscess, and 19.38% with fistula. The size of the masses was distributed as follows: <2 cm in 4.5% of patients, ≥2 to <5 cm in 67.0%, and ≥5 cm in 28.4%. The study reported a response rate of 80.7% and clinical-radiological complete remission in 47.6% of patients after SPST. The authors reported that the masses that became smaller (lesion size <3 cm) were suitable for the watch-and-wait method or partial mastectomy (20). In our study, lesion size (average mass size 5.9±2.6 cm) and the incidence of acute mastitis (98.7%), breast abscess (79.5%), and breast skin symptoms (70.5%) were higher compared to the literature studies. However, our study was similar to the literature in that complete remission was achieved in 46.2% of the patients who received systemic treatment with peroral prednisolone in the first session after clinical and pathological diagnosis. After the additional second session (83.3%) and third session (100%) for patients who showed resistance to treatment and/or had recurrence, all patients achieved complete remission, and 14.1% of the patients underwent partial mastectomy.

IGM is a disease with a heterogeneous structure and determining the risk profile for recurrence in patients before treatment is important in the management of the disease. In the literature, clinical parameters associated with recurrence include undiagnosed breast infections, smoking, low vitamin B12 levels, accompanying rheumatic diseases, erythema nodosum, OCP, history of childbirth and breastfeeding, obesity, fistula, abscess, and luminal inflammation degree (7,26-28). Velidedeoglu et al. (19) observed at least one recurrence in all patients with bilateral IGM in 2016. Following additional combined treatments, complete remission was achieved in 90% (9/10) of these patients, while the disease remained static in one patient (19). In another study conducted by the same authors in 2022, there was no relationship between disease recurrence and factors such as breastfeeding, trauma, OCP use, and smoking (6). Similarly, Çetinkaya et al. (29) did not observe a relationship between age, body mass index (BMI), OCP use, tobacco use, and preoperative platelet to lymphocyte ratio (PLR) in patients who experienced recurrence after surgical treatment. However, the pre-operative neutrophil to lymphocyte ratio (NLR) was significantly associated with recurrence in that study (29). Similarly, in Kargın et al. (30), NLR before surgical and medical treatment was significantly higher in patients with recurrence. In 2022, Ciftci et al. (16) analyzed data from 85 patients they treated using different treatment modalities. It was revealed in the study that treatment methods, BMI, parity, HIP [white blood cells, neutrophil, lymphocyte, thrombocyte, NLR, PLR), C-reactive protein (CRP)], and sedimentation values were not associated with recurrence. According to the multivariate analysis, smoking and the albumin/globulin ratio were independent risk factors for recurrence. The authors reported that CRP, sedimentation and HIP was dynamic parameters that could change daily and be influenced by body fluid balance (15). As it is seen, there are different results regarding the identification of the risk profile of the patients in the literature due to the use of various treatment modalities, different study designs, patients' hemodynamics, which are subject to daily changes, and heterogenous clinical properties of the disease. In our study, the incidence rate of abscess and side effects secondary to treatment was significantly higher ($p<0.05$) in the group of patients who showed resistance to treatment and/or experienced recurrence (53.8%) in the first session of SPST and the side effects were the limitations of SPST. Of the patients who showed resistance to treatment and/or experienced relapse, 39.7% (31 of 78) achieved remission after a combination of medical treatments and 14.1% (11 of 78) achieved remission after a combination of medical and surgical treatments.

Study Limitations

This study has some limitations. First, the study data were retrospectively collected. Second, the sample size was small due to the rarity of IGM. Third, we could not evaluate erythema nodosum, albumin, globulin, albumin/globulin ratio, and BMI because of insufficient data in patients' medical records.

Conclusion

IGM is a benign chronic disease of the breast that is characterized by heterogeneous clinical findings and may have an aggressive course

from time to time. In patients with clinical-radiological presentation of abscess and/or bilateral disease before treatment, the disease tends to have an aggressive course and the frequency of secondary side effects of systemic peroral steroid therapy is higher. Combined medical and/or surgical treatments may be required for the management of the disease in these patients. For this reason, these patients should be informed about the risk of relapse, side effects, and combined treatments, and they should be followed up more closely.

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Effect of Mannose-Binding Lectin Gene Polymorphism on Infection in Patients Undergoing Autologous Hematopoietic Stem Cell Transplantation

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ABSTRACT

Introduction: The aim of this study was to investigate the association of mannose-binding lectin (MBL), which is involved in the classical complement pathway in innate immunity, with infections in the autologous hematopoietic stem cell transplantation (AHSCT) process and the frequency of *MBL* gene polymorphism.

Methods: Single gene nucleotide polymorphisms of codons 52 and 54 in exon 1 of the *MBL-2* gene were investigated in 30 patients who had received AHSCT with a diagnosis of multiple myeloma in the Adult Bone Marrow Transplant Unit between January 2020 and December 2020. Demographic characteristics, engraftment times, and infectious processes of the patients were recorded.

Results: Neutropenic fever developed at least once in 28 (93.3%) patients during AHSCT. During hospitalization pneumonia developed in 6 (20%), urinary tract infection in 4 (13.3%), and catheter infection in 4 (13.3%) patients. *MBL* gene polymorphism at codon 54 was found in 4 (13.3%) of the patients included in the study. Among the 4 patients with *MBL* gene polymorphism, 2 had pneumonia and 2 had urinary tract infection ($p=0.16$ and $p=0.07$, respectively). There was no difference between the patients with *MBL* gene polymorphism and those without *MBL* gene polymorphism in terms of age, gender, and infection type ($p>0.05$).

Conclusion: Bacterial infection was observed in all patients with *MBL* gene polymorphism during AHSCT. This may be related to the increased susceptibility to infection caused by *MBL* gene polymorphism. However, in this study no relationship was found between *MBL* gene polymorphism and infection frequency and type in AHSCT.

Keywords: Mannose-binding lectin, autologous, transplantation

Introduction

Mannose-binding lectin (MBL) is involved in the lectin pathway of capillary activation and functions as a part of innate immunity (1,2). When MBL binds to carbohydrates on the surface of microorganisms, serine proteases of the lectin pathway are activated. Then, the lectin pathway and the classic complement pathway together from a membrane attack complex and microorganisms are lysed (3).

MBL gene polymorphisms increase the susceptibility to infection by decreasing MBL serum levels. A single gene nucleotide polymorphism is defined in exon 1 of the *MBL-2* gene. In Eurasia, codon 54 polymorphism is observed at a rate of 25%, and this is the most common MBL variant (4).

It is known that patients with MBL deficiency have increased susceptibility to infections such as *Pseudomonas* and *Meningococci* (5).

Multiple myeloma (MM) is a malignant disease characterized by an uncontrolled, clonal increase in plasma cells in the bone marrow. High-dose melphalan followed by autologous hematopoietic stem cell transplantation (AHSCT) is the standard treatment for MM in appropriate patients after induction therapy (6).

The aim of this study was to investigate the frequency of *MBL-2* gene polymorphism and its relationship with infections in the process of AHSCT.

Methods

Our study was prospectively planned. The study was carried out with the permission of the İstinye University Hospital Clinical Research Ethics Committee (approval number: 2/2019.K-019, date: 04.12.2019). All procedures were carried out in accordance with the ethical rules and principles of the Declaration of Helsinki.

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Between January 2020 and December 2020, 30 patients who were planned to undergo AHSCT with a diagnosis of MM at our hospital were included in the study after their consent was obtained. In this prospective study, 5 mL peripheral blood samples were obtained from 30 patients before transplantation to investigate single gene nucleotide polymorphisms of codons 52 and 54 in exon 1 of the *MBL-2* gene. *MBL-2* gene rs1800450 C>T, rs7095891 G>A polymorphisms were analyzed by sequencing using sequencing kits (GML, Switzerland). All patients received VCD (bortezomib, dexamethasone, cyclophosphamide) or PAD (bortezomib, adriamycin, dexamethasone) as standard induction therapy followed by AHSCT for consolidation. Ciprofloxacin 500 mg 1x1, fluconazole 200 mg 1x1, acyclovir 200 mg 3x1 and trimethoprim sulfamethoxazole 800/160 mg 2x1, 2 days a week were administered prophylactically to all patients. All patients received melphalan 200 mg/m^2 IV on day -2 before AHSCT. All patients received at least $2x10^6$/kg CD34+ AHSCT, and granulocyte colony-stimulating factor 300 µg/day IV was initiated on day 4 after stem cell infusion. Neutropenic fever was defined as an absolute neutrophil count $<0.5x10^9$/L and a temperature of 38.3 degrees Celsius or higher on tympanic measurement. Blood, catheter and urine cultures of the patients who developed neutropenic fever were taken, ciprofloxacin 500 mg 1x1 was stopped and IV broad spectrum antibiotics vancomycin and meropenem were initiated. Engraftment time was defined as a neutrophil count $\geq 0.5x10^9$/L for 3 consecutive days. Demographic characteristics of the patients, neutropenic fever status during the transplantation process, treatments received, infection type, blood culture, catheter culture, and urine culture results, and engraftment times were recorded.

Statistical Analysis

SPSS (version 20) Windows software was used for statistical analysis. Confidence interval at 95% level and $p<0.05$ were considered statistically significant. Chi-square test or if applicable Fischer's exact test was used for comparison of the groups.

Results

Among the 30 patients included in the study, 11 were female (36.7%) and 19 were male (63.3%) and the age range was 50-70 years with a mean age of 60 years. Neutropenic fever developed at least once in 28 (93.3%) patients during AHSCT. During hospitalization pneumonia developed in 6 (20%), urinary tract infection in 4 (13.3%), and catheter infection in 4 (13.3%) patients. Among the 6 patients who developed pneumonia, 2 of them developed pseudomonas and 1 of them developed haemophilus influensa in sputum culture, and prophylactic antifungal treatment was extended in 3 (10%) patients because of fungal pneumonia. *E. coli* was grown in urine culture in all patients with urinary tract infection. Serratia marcescens were grown in 2 patients and *Staphylococcus aureus* was grown in 2 patients in the catheter tip culture of patients with a catheter infection.

Neutrophil engraftment time was 10 days in 11 (36.7%), 11 days in 7 (23.3%), 12 days in 9 (30%), and 13 days in 3 (10%) patients.

CC, CT, TT for MBL rs1800450 C>T and GG, GA, AA genotypes for MBL rs7095891 G>A were analyzed. MBL rs1800450 C>T was found in the CC normal genotype in all patients, MBL rs7095891 G>A was found in GA heterozygous in 4 (13.3%) patients, and in the GG normal genotype in 26 (86.7%) patients. Among the 4 patients with *MBL* gene polymorphism, 2 had pneumonia and 2 had urinary tract infection ($p=0.16$ and $p=0.07$, respectively). No difference was found between the patients with *MBL* gene polymorphism and those without *MBL* gene polymorphism in terms of age, gender and infection type ($p>0.05$) (Table 1).

Discussion

MBL is part of innate immunity and is involved in the lectin pathway (7). In previous studies (4), *MBL* gene polymorphism was defined as around 25%. In our study, MBL codon 54 polymorphism was found with a rate of 13.3%, whereas MBL codon 52 polymorphism was not found in any patient. 4 (13.3%) patients with MBL rs7095891 G>A polymorphism was in the heterozygous (GA) genotype.

Table 1. Descriptive and clinical characteristics of the groups

Parameter	G54D (+), (n=4)	G54D (-) and R52C (-), (n=26)	p-value
Age	59±9	62±8	0.24
Gender			
Female	2 (50%)	9 (34.6%)	0.61
Male	2 (50%)	17 (65.4%)	
Infection type			
Pneumonia	2 (50%)	4 (15.4%)	0.16
Bacterial	2 (50%)	1 (3.8%)	
Pseudomonas	2 (50%)	0	
Haemophilus influensa	0	1 (3.8%)	
Fungal	0	3 (11.6%)	
Urinary tract infection	2 (50%)	2 (7.7%)	0.07
E. coli	2 (50%)	2 (7.7%)	
Catheter infection	0	4 (15.4%)	
Serratia marcescens	0	2 (7.7%)	
Cytaphylococcus aureus	0	2 (7.7%)	

In previous studies (8,9), *MBL* gene polymorphism increases susceptibility to some infections in the process of stem cell transplantation. In our study, pneumonia or urinary tract infection was observed in all patients with *MBL* gene polymorphism during AHSCT. In addition, pneumonia was observed in 15.4% and urinary tract infection in 7.7% of patients without *MBL* gene polymorphism. This difference between groups was not statistically significant. This may be related to the detection of *MBL* gene polymorphism in only 4 patients in our study.

Unlike our study, a previous study (10) showed that *MBL* gene polymorphism was associated with an increased risk of fungal infection during stem cell transplantation. Although *MBL* gene polymorphism was found in 2 patients with fungal pneumonia in our study, the increased risk of fungal infection with *MBL* gene polymorphism could not be demonstrated in this study because *MBL* gene polymorphism was not found in 2 patients with fungal pneumonia. This may be related to the small number of patients.

Previous studies (11) have found an increased risk of bacterial infection during the process of stem cell transplantation in patients with *MBL* gene polymorphism. Similarly, the bacterial infection was observed in all patients with *MBL* gene polymorphism in our study. Similar to previous studies (12), *MBL* gene polymorphism was in 2 patients with pseudomonas infection. This supports the increased frequency of pseudomonas infection, especially with *MBL* gene polymorphism.

Serratia marcescens and *Staphylococcus aureus* were grown in catheter cultures of 2 patients without *MBL* gene polymorphism.

E. coli growth was detected in the urine culture of 2 patients with *MBL* gene polymorphism and no resistant microorganisms were grown in the urine culture of any of the patients.

All patients were administered prophylactic oral acyclovir 600 mg/day during AHSCT. Unlike a previous study (13), in our study, no difference was found in terms of viral infections in patients with or without *MBL* gene polymorphism. This may be related to the small number of patients.

No difference was found between patients with or without *MBL* gene polymorphism in terms of infections and response to treatments. Mortality was not detected in any patient.

Although increased infections are known to prolong engraftment time, engraftment time was not different from that expected in patients with or without *MBL* gene polymorphism.

MBL gene polymorphisms increase the susceptibility to infection by decreasing MBL serum levels (14). In our study, a heterozygous mutation in codon 54 of the *MBL-2* gene was detected in 4 (13%) patients. Since MBL serum levels of the patients were not analyzed, no comparison could be made regarding MBL serum levels.

Study Limitations

The most important limitation of this study is the small number of patients. In addition, the lack of comparison with MBL serum levels and the absence of a control group are other limitations.

Conclusion

In this study, bacterial infection was observed in all patients with *MBL* gene polymorphism during AHSCT. This may be related to the increased susceptibility to infection caused by *MBL* gene polymorphism. However, in this study no relationship was found between *MBL* gene polymorphism and infection frequency and type in AHSCT. Considering the number of patients in this study, in patients who are planned to receive intensive immunosuppressive treatment such as stem cell transplantation, it may be an appropriate approach to investigate *MBL* gene polymorphisms in patients with low MBL serum levels. In this way, close follow-up of infections, differentiation of prophylactic agents to be used, and effective treatment of infections may be possible in this group of patients during the processes requiring intensive immunosuppressive treatment. The results of this study need to be supported by randomized prospective, clinical, laboratory, and histopathological studies with a larger number of patients.

Ethics Committee Approval: The study was carried out with the permission of the İstinye University Hospital Clinical Research Ethics Committee (approval number: 2/2019.K-019, date: 04.12.2019).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

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Clinical and Radiological Outcomes of Two-Stage Revision Knee Arthroplasty in Infected Primary Knee Arthroplasty

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ABSTRACT

Introduction: Two-stage revision arthroplasty is the gold standard treatment for infected knee arthroplasty. The primary objectives of this treatment approach are to eradicate the infection and restore a pain-free and well-functional joint. In this retrospective study of case series from a single center, we aimed to evaluate the clinical and radiological results of patients who underwent two-stage revision knee replacement with the diagnosis of infected knee prosthesis and share the results.

Methods: The data of patients between 2011 and 2016 were analyzed in this retrospective study. Twenty-four patients who were followed up for at least six months were included in the study. Infection markers, Knee Society Score (KSS), pain scores, range of motion (ROM), and flexion contractures were recorded before and after treatment. Radiologically, changes in the patellar tendon length, the Insall-Salvati (IS) ratio, and the joint line (JL) were evaluated.

Results: Sixteen female and eight male patients with a mean age of 68.0±8.6 years were studied. The patients were followed up for 31.0±18.9 months on average. The mean clinical KSS of the patients before and after treatment was 44.7 and 76.3, respectively, while the functional KSSs were 31.7 and 63.5, respectively. The patients had a mean ROM of 60.5° and 84.8°, pain score of 8 and 2.25, and knee flexion contracture of 1.38° and 0.21° before and after the treatment. Pre- and post-treatment IS and JL values did not have a statistically significant effect on the clinical and functional outcomes.

Conclusion: The early- tomid-term results of patients who underwent two-stage knee revision arthroplasty were satisfactory in terms of clinical and functional results. Postoperative JL position and IS ratio had no significant effect on functional outcomes. The use of dynamic spacers and the short time between two stages had a positive effect on the results.

Keywords: Infected knee arthroplasty, joint line, Knee Society Score, two-stage revision

Introduction

Due to the increase in the elderly population in parallel with the prolongation of life expectancy and high success rates, total knee arthroplasty is one of the most frequently performed orthopedic elective surgeries. It is estimated that the number of total knee replacements in the United States will increase by 85% by 2030 (1). Notably, instability, mechanical loosening, malposition of the prosthesis, dislocation, polyethylene abrasion, periprosthetic fractures, and infection are the predominant complications necessitating revision surgery after total knee replacement (2).

Infection, which has a prevalence of 0.5-2% after total knee replacement, draws attention as the most important complication with a long and costly treatment (3,4). It is anticipated that the increase in the estimated total number of knee replacements will also increase the number of patients with infections, which in turn will create a serious burden on the health system and the economy (5,6). Various treatment options are available for infected knee prostheses, including irrigation and debridement, one or two-stage revision arthroplasty, arthrodesis, amputation, and antimicrobial suppression without surgical intervention (7). The primary objectives of treatment encompass eliminating the infection and restoring a pain-free and well-functional joint. Two-stage revision arthroplasty is the gold standard in the treatment where the above goals are aimed (8-10). The first stage of the two-stage revision arthroplasty includes removing infected components, extensive debridement and

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tissue sampling, placing an antibiotic-loaded dynamic or static spacer into the joint followed by appropriate antibiotic treatment for the infection, and if necessary re-debridement to eradicate the infection, while revision procedures are performed in the second stage (11).

In this retrospective study of case series from a single center, we aimed to evaluate the clinical and radiological results of patients who underwent two-stage revision knee replacement with the diagnosis of infected knee prosthesis and share the results.

Methods

The data of patients who underwent two-stage knee revision arthroplasty with the diagnosis of an infected knee prosthesis in our clinic between 2011 and 2016 were retrospectively analyzed. Of the 28 patients identified, 24 who were diagnosed with an infection after primary knee arthroplasty were regularly followed up for at least six months, and patients whose laboratory tests and radiological images could be accessed were included in the study. Patients with a diagnosis of infected revision arthroplasty, who underwent arthrodesis or one-stage revision surgery, and without regular follow-ups were excluded from the study. The study was approved by University of Health Sciences Turkey, İstanbul Training and Research Hospital Institutional Ethics Committee (approval number: 936, date: 03.02.2017).

The physical examination findings of the patients were evaluated together with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell levels, which are infection indicators. Microbiological studies were performed by aspiration of joint fluid from the patients. Standard radiographs were checked for septic loosening. Two-stage revision arthroplasty was planned for patients with confirmed infection according to Musculoskeletal Infection Society criteria (12). In the first stage, the implants were removed and extensive debridement was performed. Then, perioperative joint fluid and tissue samples were sent for culture. The first stage was completed by placing a dynamic or static spacer into the joint cavity with antibiotic-loaded cement, both for the treatment of infection and to obtain a functional joint after the treatment. After the first stage, specific antibiotic therapy was started for patients whose preoperative cultures had the causative microorganism. Broad-spectrum antibiotic therapy was initiated as empirical treatment for patients whose cultures did not yield any microorganisms. After the perioperative culture was concluded, appropriate antibiotic treatment was planned for all patients in consultation with the infection clinic. The patients received at least six weeks of intravenous and oral antibiotics. Between the two stages, the patients were allowed controlled joint movement with the use of an angle-adjustable brace. During antibiotic therapy, the CRP and ESR levels of the patients were monitored. A second stage was planned for revision arthroplasty for patients who showed no signs of infection and whose CRP and ESR levels were significant or regressed to normal. In the second stage, the spacer and cement were removed. Debridement, culture sampling, and synovial cell count were repeated during the operation, and revision arthroplasty was performed. After completion of the second stage, appropriate antibiotic therapy was administered empirically until the culture results were obtained. Subsequently, the patients were followed-up in the postoperative period using the same laboratory parameters.

The patients were evaluated both before and after treatment with the American Knee Society's clinical and functional scoring system [Knee Society Score: (KSS)] (13). Numerical pain scores, joint range of motion (ROM), flexion contractures, and complications were also recorded before and after treatment.

Patients were evaluated radiologically according to changes in the patellar tendon length (PTL), the Insall-Salvati (IS) ratio, and joint line (JL) before and after revision arthroplasty. On the lateral knee X-ray, the distance between the lower pole of the patella and the tibial tubercle was defined as PTL, the ratio between PTL and patella length was defined as IS ratio, and the distance between the head of the fibula and the lateral femoral condyle was defined as JL.

Statistical Analysis

SPSS 15.0 for Windows software was used for statistical analyzes. Descriptive statistics are given as the mean, standard deviation, minimum, and maximum for the numerical variables and as numbers and percentages for the categorical variables. Comparisons of the numerical variables without normal distribution in two independent groups were made using Student's t-test, whereas those with normal distribution were compared using the independent Mann-Whitney U test. The paired samples t-test was used when the differences of the numerical variables in dependent groups met the normal distribution criteria, whereas the Wilcoxon test was employed when the criteria were not met. The relationships between the numerical variables were analyzed with Pearson's correlation when the parametric test conditions were met and with Spearman's correlation when the conditions were not met. The difference in ratios in independent groups was analyzed by the chi-square analysis. The statistical significance level was set at $p<0.05$.

Results

Of the 24 patients included in the study, 16 (67%) were females and eight (33%) were males, with a mean age of 68.0±8.6 years (range: 46 to 82 years). The mean follow-up period was 31.0±18.9 months (range: 6 to 65 months). The cultures of 13 patients (54.2%) did not grow any microorganisms. In the cultures of the remaining 11 patients, *S. epidermidis* was observed in three (12.5%), MRSA in two (8.3%), *E. coli* in one (4.2%), *Pseudomonas aeruginosa* in one (4%, 2), *S. aureus* in one (4.2%), *Serratia marcescens* in one (4.2%), *S. haemolyticus* in one (4.2%) and *Enterococcus* + *S. epidermidis* in one (4.2%).

The mean ROM of the patients was 60.5°±15.0° (range: 0° to 80°) before the treatment and 84.8°±12.9° (range: 45° to 100°) after the revision arthroplasty. While the mean flexion contracture was measured at 1°±3.2° (range: 0° to 10°) before the treatment, it was measured at 0.2°±1.0° (range: 0° to 5°) after the revision arthroplasty. The mean numerical pain score before treatment was 8.0±1.3 (range: 6 to 10), whereas the score at the end of the follow-up was 2.3±1.3 (range: 0 to 5). The differences between the ROM, flexion contracture, and pain measurements before and after the treatment were statistically significant ($p<0.001$) (Table 1).

The mean duration of antibiotic use between the two stages was 10.8±7.4 weeks. When the duration of antibiotic use between the two stages of the patients and the KSSs were compared, a negative but insignificant correlation was found (Table 2).

During the first phase, the joint space was filled using dynamic spacers in 15 patients and static spacers in nine. We noted that the patients in whom dynamic spacers were used had higher ROM and clinical and functional KSSs after revision arthroplasty than those in whom static spacers were used. However, the difference was not statistically significant (p=0.676, p=0.232, and p=0.630, respectively) (Table 3).

The clinical and functional KSSs of the patients were evaluated before and after treatment. The mean clinical KSS before treatment was 44.7±10.6, while it improved to 76.3±10.4 at the end of the follow-up. Accordingly, the results of 23 patients (95.8%) were "poor" and one patient (4.2%) was "moderate" before the treatment, whereas the results were "excellent" in 10 patients (41.7%), "good" in eight (33.3%), and "moderate" in six (25%). The mean functional KSS was 31.7±17.2 (range: 0 to 60) before the treatment, whereas it was 63.5±20.1 (range: 20 to 90) at the end of the follow-up. The differences between the pre- and post-treatment measurements of the clinical and functional KSSs were statistically significant (p<0.001).

There was no statistically significant difference in the mean PTL, IS ratio, and JL measurements before and after revision arthroplasty (Table 4). The relationship between the IS ratio and JL measurements obtained from the knee radiographs of the patients after revision arthroplasty and the clinical and functional KSSs at the end of follow-up was also evaluated and no statistical significance was found (Table 4).

No additional complications were encountered in the patients during the first phase of the spacer application and until the pre-revision period. However, reinfection was observed in two patients (8.3%), knee instability in one (4.2%), and wound site infection in one (4.2%) during and after revision surgery.

Table 1. ROM, flexion contracture, and pain measurement results before and after the revision surgery

		Mean ± SD	p
ROM (°)	Pre-revision	60.5±15.0	<0.001*
	Post-revision	84.8±12.9	
Flexion contracture (°)	Pre-revision	1.4±3.2	0.068†
	Post-revision	0.2±1.0	
Pain score	Pre-revision	8.0±1.3	<0.001†
	Post-revision	2.3±1.3	

ROM: Range of motion, SD: Standard deviation, *Paired t-test, †Wilcoxon test

Table 2. Statistical relationship between the duration of antibiotic use and the clinical and functional KSSs after revision arthroplasty

	Duration of antibiotic therapy (weeks)	
	r	p*
Clinical KSS after revision	-0.367	0.078
Functional KSS after revision	-0.265	0.212

r: Correlation coefficient, KSS: Knee Society Score, *Spearman's correlation

Table 3. The relationships among the ROM and the clinical and functional KSSs after revision arthroplasty according to the type of spacer used

	Spacer type				p*
	Dynamic (n=15)		Static (n=9)		
	Mean ± SD	Median	Mean ± SD	Median	
ROM after revision	87.1±9.3	88.0	80.9±17.2	85.0	0.676
Clinical KSS after revision	78.1±10.5	80.0	73.3±10.1	74.0	0.232
Functional KSS after revision	65.3±20.2	65.0	60.6±20.7	60.0	0.630

KSS: Knee Society Score, ROM: Range of motion, SD: Standard deviation, *Mann-Whitney U test

Table 4. The mean PTL, IS ratio, and JL values and their statistical relationships before and after revision arthroplasty and the correlation of post-revision JL and IS values with the clinical and functional KSSs

		Mean ± SD		Min.-Max.	p
PL (cm)	Preoperative	4.41±0.56		3.05-5.20	0.322*
	Postoperative	4.28±0.75		3.25-6.00	
IS	Preoperative	1.10±0.22		0.74-1.45	0.127*
	Postoperative	1.04±0.17		0.76-1.40	
JL (cm)	Preoperative	1.04±0.41		0.38-1.95	0.182*
	Postoperative	1.21±0.57		0.51-2.71	
		JL after revision		IS after revision	
		r	p†	r	p†
Clinical KSS after revision		-0.217	0.359	-0.106	0.658
Functional KSS after revision		-0.286	0.222	-0.212	0.368

IS: Insall-Salvati ratio, JL: Joint line, KSS: Knee Society Score, Min-Max: Minimum-Maximum, PL: Patellar length, r: Correlation coefficient, SD: Standard deviation, *Paired t-test, †Spearman's correlation

Discussion

The aim of revision surgeries after infected knee arthroplasty is to obtain a painless and functional knee joint where the infection is eradicated. In our study, the results obtained after revision knee arthroplasty performed in two stages met these goals.

The debate is still on whether one- or two-stage revision arthroplasty should be performed after infected total knee replacement. Although a higher reinfection rate was reported in one-stage revision arthroplasty (0-11%) in a systematic evaluation compared to two-stage revisions (0-40%), the superiority of the two procedures over each other was not proven due to the lack of studies with sufficient evidence (14). In another systematic evaluation by Nagra et al. (15), the authors emphasized that one-stage revision surgery has better clinical results and lower reinfection rates in selected patients. Similarly, a recent systematic review showed no statistical difference between functional outcomes and eradication rates in one- or two-stage treatment (4). The largest case series study in the literature with a two-stage procedure was conducted in 2012 by Mahmud et al. (16). The authors performed two-stage revision surgery on 253 knees with an average follow-up of 48 months and observed reinfection in 16 patients (7%). The authors also reported that infection-free time after two-stage revision surgery was five years in 85% and 10 years in 78% of their patients. In our study, reinfection developed in two (8.3%) of our patients who were followed up for an average of 31 months. The clinical KSSs before and after the treatment were 44.7 and 76.3, and the functional KSSs were 31.7 and 63.5, respectively.

In a comparative study, Park et al. (17) used antibiotic-loaded static spacers in 20 knees and dynamic spacers in 16 knees and reported better functional scores and wider ROM in patients in whom dynamic spacers were used with no increase in reinfection rate and bone loss. In a systematic review published by Voleti et al. (18), a total of 1,526 patients who used static spacers in 654 knees and dynamic spacers in 872 knees were examined. There was no significant difference between the two groups in terms of reinfection. However, after the second stage, a significant difference in ROM was observed, especially in the group that used dynamic spacers. In addition, no significant difference was found in terms of clinical scores and wound-related complications (17). In our study, the ROM and clinical and functional KSSs were also higher after revision in patients to whom dynamic spacers were applied. The post-revision results of both groups support the literature and are satisfactory.

To obtain adequate alignment and function in both primary total knee prosthesis and revision knee prosthesis, kinematic reconstruction should be well understood (19-21). The importance of the JL and the restoration of patellar height in revision knee arthroplasty has been the subject of several publications. Malposition of the JL causes decreased extensor strength, patellar impingement syndrome, anterior knee pain, patellar instability, and decreased ROM (21-23). In revision knee arthroplasty, severe bone loss and changes in soft tissue pose a challenge for orthopedic surgeons in restoring the JL, adjusting the patellar height, and providing knee stability. The augments in modern revision arthroplasty systems have been the solution for restoring the JL by restoring the distal femoral bone loss (21). In a study evaluating the JL and patellar height after revision knee arthroplasty, 74 knees of 70 patients were examined (24). Forty-seven knees had to undergo a two-stage revision and 27 knees one-stage revision due to aseptic loosening. The mean JL lengths of all patients increased from 17.51 mm to 18.37 mm, while the IS ratio decreased from 0.98 to 0.92 and PTL from 42.92 mm to 39.45 mm. The authors observed that the JL and IS ratio had greater changes in the septic group. In addition, functional results were lower in the septic group than in the aseptic group. Finally, the researchers stated that the JL position and IS ratio did not correlate with functional scores. The values we found for the JL, IS ratio, and PTL were in similar ranges with the studies in the literature, and similarly there was no correlation between these values and post-treatment functional scores.

Study Limitations

Its retrospective design, the low number of cases, the lack of a control group, and the fact that it included a single surgical procedure can be considered the limitations of our study. The literature should be supported by comparative studies with a larger number of cases.

Conclusion

Two-stage revision arthroplasty after septic knee arthroplasty is a satisfactory intervention in terms of clinical and functional results. The use of dynamic type spacers and the short time between the two stages have a positive albeit statistically insignificant effect on the results.

Ethics Committee Approval: The study was approved by University of Health Sciences Turkey, İstanbul Training and Research Hospital Institutional Ethics Committee (approval number: 936, date: 03.02.2017).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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Experience in Non-invasive Ventilation in Grade 3 Hepatic Encephalopathy

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ABSTRACT

Introduction: Hepatic encephalopathy (HE) is a common complication of acute and chronic liver failure, which can lead to significant morbidity and mortality. Grade 3-4 HE patients are typically managed with routine intubation and mechanical ventilation. However, recent research suggests that routine intubation may increase the risk of complications and death for the preservation of the airway. This study investigated the use of non-invasive ventilation (NIV) in patients with grade 3 encephalopathy associated with acute and chronic liver failure.

Methods: This retrospective study included patients with grade 3 encephalopathy associated with liver failure who underwent NIV between January 2022 and March 2023 in a liver transplant intensive care unit. The patient demographic data, laboratory results, comorbidities, and outcomes were collected and analyzed. The results were compared to those of HE patients who were intubated as reported in the literature.

Results: A total of 41 children and adults with grade 3 HE who received NIV were included in this study. Compared with HE patients who were intubated as reported in the literature, the NIV group had significantly lower rates of complications and mortality. Additionally, there were no additional complications observed in patients who received NIV without intubation, such as infections, cardiovascular disorders, or cognitive impairments.

Conclusion: The use of NIV in grade 3 HE patients suggests that it is an effective alternative to intubation. These findings support the need for careful consideration when deciding to intubate HE patients and suggest that continuous support using NIV may provide potential benefits. Further studies are needed to investigate the optimal management of these patients to improve their outcomes.

Keywords: Hepatic encephalopathy, hepatic failure, non-invasive ventilation

Introduction

Hepatic encephalopathy (HE) is the critical stage of liver failure, especially in acute cases. Patients with grade 3 or 4 HE is often intubated and underwent mechanical ventilation to prevent life-threatening complications (1,2). However, there is no concrete evidence that routine intubation has benefit for patients with advanced delirium associated with HE (3,4). In contrast, this procedure has its own various risks, such as nosocomial pneumonia, hypotension, increased mortality, and prolonged hospital stays (5,6). Thus, mechanical ventilation in patients with grade 3-4 HE has been associated with increased in-hospital mortality due to compromised immunity, altered drug metabolism, and circulatory dysfunction (7,8). The aim of this study was to determine the effects of preferring non-invasive ventilation (NIV) instead of intubation in patients with grade 3 HE in the aspect of clinical outcome.

Methods

In this study, the records of patients followed in the University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital between January 2022 and March 2023 were retrospectively reviewed. Patients with grade 3 HE related to acute or chronic hepatic failure to respiratory impairment due to cardiovascular insufficiency were included in the study. All these patients underwent NIV and continued to receive extracorporeal liver support therapy (9). "Acute Physiology and Chronic Health Evaluation (APACHE II)", "West Haven Classification" and "Glasgow Coma Scale" were used to stage the clinical conditions. The ethical rules of this study were determined in accordance with the Declaration of Helsinki. All necessary precautions were taken to ensure the confidentiality and protection of data.

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This study was approved by the Ethics Committee of University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital (approval number: 336, date: 26.07.2023).

Statistical Analysis

Statistical analyzes were performed using SPSS 20 (IBM Corp., Armonk, NY). The normal distribution of study data was evaluated using the Kolmogorov-Smirnov analysis. Demographic characteristics, intensive care unit (ICU) length of stay, APACHE and PRISM scores, and NIV parameters of the patients were presented as median (range), while laboratory values at admission to the ICU were presented as mean (standard deviation).

Results

A total of 41 patients with grade 3 HE were included. Of these patients, 24 were female and 17 were male. Thirteen of the patients were children with a mean age of 8 (range: 0-16 years), while the mean age of the 28 adult patients was 57 (range: 49-70) years. NIV was applied to the 28 adult patients for a mean of 8 days (range: 3-13 days) and to the 13 pediatric patients for a mean of 9 days (range: 4-14 days) until recovery, liver transplantation, or progression to grade 4 HE occurred. The application was performed for 20 hours per day. Nine patients (22%) progressed to grade 4 HE due to a rapid increase in hepatic failure and required protracheal intubation. These nine patients who were not suitable for liver transplantation or could not find a suitable donor died. Recovery was achieved in the other 24 patients who received NIV, and no additional complications such as nosocomial pneumonia, respiratory disorders, hypotension, or cognitive impairment were observed. Eight other patients who received NIV underwent liver transplantation and achieved recovery. The demographic, etiological and intensive care scoring values of the patients are presented in Table 1, the NIV values in Table 2 and the laboratory values in Table 3.

Table 1. Demographics, etiology and intensive care scoring values

Child	**13 (n)**
*Age	8 (0-16)
*PRISM score	29 (22-36)
Adult	**28 (n)**
*Age	57 (49-70)
*APACHE II score	25 (20-28)
Gender	**n**
Male	17
Female	24
Etiology	**n (%)**
Mushroom intoxication	11 (26.8)
Autoimmune disease	5 (12.1)
Toxic hepatitis	5 (12.1)
Hepatitis B	4 (9.7)
Wilson's disease	4 (9.7)
Paracetamol intoxication	3 (7.3)
Idiopathic disease	3 (7.3)
Progressive familial intrahepatic cholestasis	2 (4.8)
Alveolar hydatid cyst	2 (4.8)
Budd chiari syndrome	1 (2.4)
Wolcott-Rallison syndrome (WRS)	1 (2.4)

*Median values (range), APACHE-II: Acute Physiology and Chronic Health Evaluation-II, PRISM: Pediatric Risk of Mortality

Discussion

HE, which worsens to grade 3-4 is typically managed with intubation and mandatory mechanical ventilation according to literature and algorithms (10). However, studies have shown that routine intubation for airway protection in patients with grade 3-4 HE may potentially be related to increased risk of complications and in-hospital mortality (11-13). In this study, survival rates of 78% and 22% were determined in 41 patients.

In this study, the expected complications of intubation, such as nosocomial infections, cardiovascular and cognitive disorders, and increased mortality were not seen in our patients undergoing NIV who had different demographic, etiological, mortality scoring, and laboratory results. The most remarkable and often cited complication of intubation, aspiration pneumonia, was observed to be prevented.

The data in the literature about the outcomes of NIV in patients with HE are very limited. Our study showed that NIV in HE patients could significantly reduce complications and mortality compared with the reported outcomes for intubated patients. The results of a recent study written by Saffo and Garcia-Tsao (14) support our results although our study excludes grade 4 HE. According to this comprehensive study, 40% of HE patients who were intubated within the first 48 h died in the hospital, while 19% of those who were not intubated died overall. The mortality rate for intubated patients reached 70% after the first

Table 2. Non-invasive ventilation values

NIV parameter	**Adult (range)***	**Child (range)***
PEEP (cmH_2O)	8 (6-10)	6 (4-8)
P support (cmH_2O)	14 (10-16)	8 (6-12)
FiO_2	0.40 (0.35-0.55)	0.40 (0.21-0.5)
NIV duration (days)	8 (3-13)	9 (4-14)
SpO_2 (%)	99 (97-100)	99 (97-100)

PEEP: Positive end-expiratory pressure, P support: Support pressure, FiO_2: Fraction of inspired oxygen, NIV: Non-invasive ventilation, SpO_2: Peripheral oxygen saturation
*Mean values

Table 3. Laboratory values by grade 3 of encephalopathy (child + adult)

	Child*	**Adult***
AST (IU/L)	2,250±1,356	6,316±3,367
ALT (IU/L)	2,158±1,285	5,929±4,423
T. Bil (mg/dL)	35±11.2	21.2±11.5
INR	3.21±0.64	4.2±0.6
Procalcitonin (ng/mL)	13.4±10.1	11±13.2
CRP (mg/L)	98±7.1	101±29.3

*Mean ± SD: Mean ± Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T. Bil: Total bilirubin, INR: International normalized ratio, CRP: C-reactive protein

2 days. In the same study, hospital stay was found to be statistically significantly longer for intubated patients compared with those who were not intubated.

The mechanism of complications that occur in intubated patients with HE has a defined physiological basis, which is circulatory, neurological, and immune dysfunction seen in intubated patients (15). Endotracheal intubation can worsen cardiovascular, cognitive, and immune function due to its increased risk of shock, delirium, infection, and other complications in patients with liver failure (16,17).

Study Limitations

The exclusion of patients with grade 4 HE is the main limitation of this case series. Therefore, we compared our results with the outcomes of intubated HE patients reported in the literature (14,18). We also did not have any patients in our center with HE who were not underwent with non-invasive or invasive respiratory support. This is an understandable situation because our ICU is a part of a liver transplantation center and not an ICU for general supportive aims.

Conclusion

This study supports the use of NIV instead of intubation for grade 3 HE patients with acute and chronic liver failure. The lower risk of complications and mortality of NIV highlights the need for careful consideration when deciding whether to intubate grade 3 HE patients. However, the lack of a control group undermines our courage to declare the need for more detailed retrospective or prospective studies to improve the optimal management of these patients before making definitive clinical recommendations.

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Ethics Committee Approval: This study was approved by the Ethics Committee of University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital (approval number: 336, date: 26.07.2023).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - İ.O., M.Ç.; Concept - İ.O., M.Ç.; Design - İ.O.; Data Collection or Processing - İ M.Ç., E.K.; Analysis or Interpretation - İ.O., M.Ç., E.K.; Literature Search - M.Ç., E.K.; Writing - İ.O., E.K.

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Evaluation of Potentially Inappropriate Drug Use in Older Adult Outpatients Using the Turkish Inappropriate Medication Use in the Elderly Criteria in Kütahya Province, Turkey

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ABSTRACT

Introduction: The population aged 65 years and older has the highest rates of drug use and is more sensitive to drug effects. Appropriate medication use among older adults is associated with poor clinical outcomes. The objective of this study was to use national criteria to evaluate inappropriate drug use among individuals aged 65 years and older who were admitted to the internal medicine outpatient clinic.

Methods: Our study was conducted using 385 randomized patients aged 65 years and older who were treated at the Evliya Çelebi Training and Research Hospital, Internal Medicine Outpatient Clinic of Kütahya University. The study was designed as a cross-sectional descriptive survey. It evaluated potentially inappropriate drugs and possible prescribing neglect according to the Turkish Inappropriate Medication Use in the Elderly (TIME) criteria.

Results: Potentially inappropriate drug use was detected in 127 patients (33%) using the TIME-to-STOP criteria. The first three potentially inappropriate medications determined by the TIME-to-STOP criteria were proton pump inhibitor deficient use, tight blood pressure control, and tight blood sugar control. Using the TIME-to-START criteria, potential prescribing omissions (PPO) were detected in 379 (98.4%) patients. The first three PPO in the TIME-to-START criteria were about vaccines deficient in patients.

Conclusion: Country-specific criteria are more effective for inappropriate medication use.

Keywords: Aged, disease, potentially inappropriate medications, inappropriate prescribing, Turkey

Introduction

Multimorbidity and polypharmacy are the main challenges faced by aging populations globally. With an aging world population, the fight against inappropriate medication use (IMU), polypharmacy, adverse drug events (ADEs), adverse drug reactions (ADRs) and medication costs has gained importance. Polypharmacy and the prescription of potentially inappropriate medications (PIMs) are the major elements of IMU in older adults. Attempts have been made to combat these issues using various explicit criterion lists (1). Potentially inappropriate prescriptions (PIP) comprise the prescription of PIMs and potential prescribing omissions (PPOs).

For older adults, this is a practical and cost-effective solution for adverse medications. In recent years, tools based on explicit PIM criteria have been developed to assist drug management in older adults for practical use in clinics.

These criteria are of great importance to prevent morbidity and mortality from ADRs and ADEs. Polypharmacy and PIMs are very common in older adults. Therefore, new strategies are needed to improve prescription quality and increase prescription safety. A review of the studies over the last 30 years indicates that criteria-based strategies have been established to prevent polypharmacy, including 42 prescription evaluation tools (2).

The criteria used for detection of PIMs and PPOs are divided into two groups, implicitly or explicitly. Although they are useful research tools, implicit criteria are not preferred in practical applications due to their complex structure and time consumption.

However, explicit criteria are useful research tools that are used in routine clinical practice. The American Geriatrics Society (AGS) Beers Criteria and the screening tool of older people's prescriptions (STOPP) and screening tool to alert to right treatment (START) criteria have been the most popular. In the 1990s, studies on PIPs using the Beers Criteria increased daily.

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The biggest reason for the diversity in these criteria is the differences in national prescribing habits. The STOPP/START criteria developed in Europe are widely used in drug reviews of older adults. It was first published in 2008 and then updated in 2015. It contains examples of PIM (STOPP) and PPO (START) separated by physiological systems. In the coming years, seniors' drug reviews will be mostly electronics. These electronic STOPP/START criteria will be administered by specialist pharmacists and will apply to lists of concurrent multimorbidity and associated polypharmacy in older adults (3).

The Beers Criteria are commonly used to evaluate PIPs in older adults. It was first published in 1991 and has been updated 5 times thus far. The last update was published in 2019. AGS has been updating the Beers Criteria since 2011. By reducing IMUs and ADEs, intended by the Beers criteria, improving the quality of care, reducing drug costs, and improving the care of older adults, it aims to reduce IMUs and ADEs by educating doctors and patients on appropriate pharmacotherapy (4).

The Turkish Inappropriate Medication Use in the Elderly (TIME) criteria were originally derived from the STOPP/START version2 and CRIME (Criteria for assessing appropriate Medication use among older adults complex patients) criteria. As a result, 55 criteria were taken from the first criteria, 17 were removed and 60 were changed. Thus, 112 TIME-to-STOP and 41 TIME-to-START criteria were established, making 153 TIME criteria (5).

The current study evaluated the PIM and PPO distributions of TIME criteria specific to Turkey.

Methods

Setting, Design, and Study Population

This descriptive prospective cross-sectional study was conducted on patients aged over 65 years. It included 385 patients who were evaluated between February 25, 2020 and December 31, 2020 in the Internal Medicine Outpatient Clinic of Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital.

The sample size was calculated as 385 individuals with 50% prevalence and 0.05 (95% confidence) significance level in an infinite population. The participants included in the study were selected using a simple random sampling method. Individuals aged over 65 years and willing to participate were included in the study. The selection was carried out separately for the morning and afternoon groups (6). Because separate appointment groups were examined in the outpatient clinics both in the morning and afternoon, patients from the two groups were included. Patients younger than 65 years of age and those who did not cooperate with the doctor during the examination were not included in the study. According to the data obtained from the IT Information Technology Department of the hospital, 2,752 patients were admitted within the specified period. A total of 385 of the 2,752 patients who visited our clinic were then randomized (Figure 1). Only the first visit examinations were evaluated between the study dates of the participants, but the follow-up examinations were not evaluated. Data were obtained through face-to-face interviews with the participants.

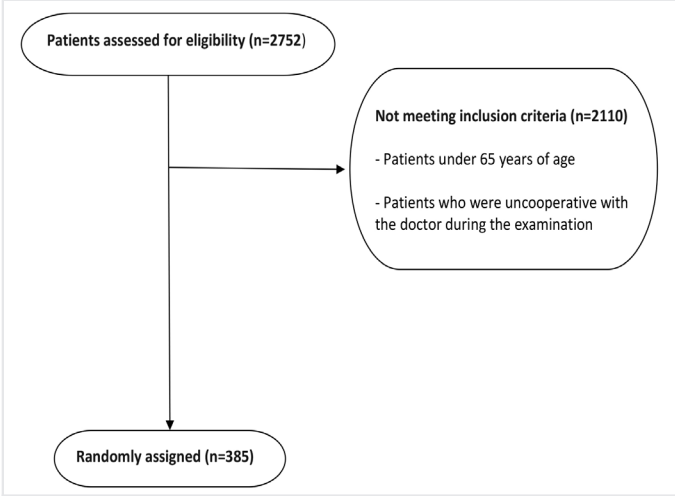

Figure 1. Flowchart of participant selection

Ethics Committee Approval

Ethics committee approval of our study was given by the Kütahya Health Sciences University (Turkey) Non-Invasive Clinical Research Ethics Committee (approval number: 2020/04-15, date: 25.02.2020). All volunteers were given oral information and provided informed consent. We carried out all stages of the study in accordance with the Declaration of Helsinki. We followed the guidelines in the "Helsinki Statement", "Guidelines for Good Medical Practices" and "Guidelines for Good Laboratory Practices".

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 22 (IBM SPSS, Turkey). Baseline characteristics and agreement with TIME recommendations were analyzed using descriptive statistics. The suitability of the study data to a normal distribution was evaluated using the Kolmogorov-Smirnov test. The results showed that the study data did not show normal distribution. We compared quantitative data and descriptive statistical methods using the Mann-Whitney U test and Wilcoxon sign test. Furthermore, we evaluated PIMs and PPOs using TIME criteria. We calculated the number of patients with PIMs and PPOs (%) and the total number of PIM and PPO cases in the total study population.

Results

A total of 385 patients aged over 65 years were enrolled in this study. Among them, 124 (32.2%) were male and 261 (67.8%) were female. Of the 385 older adults included, 73.2% were between the ages of 65 and 74 years and 26.8% were aged over 75 years. Data on the demographic distribution are presented in Table 1.

The participants in our study who met at least one relevant criterion were considered to have PIMs or PPOs. PIM was detected in 127 (33%) patients according to the TIME-to-STOP criteria. The first three PIMs determined by the TIME-to-STOP criteria were proton pump inhibitor (PPI) deficient use (9.6%), tight blood pressure control (3.1%), and tight blood sugar control (2.3%) in high-risk patients (Table 2). PPOs were detected in 379 (98.4%) patients according to the TIME-to-START criteria. The first three PPOs in the TIME-to-START criteria (herpes zoster,

Table 1. Data of demographic distribution		n (%)
Age group	65-74 years	282 (73.2)
	≥75 years	103 (26.8)
Sex	Female	261 (67.8)
	Male	124 (32.2)
Marital status	Married	244 (63.4)
	Single	1 (0.3)
	Widowed	137 (35.6)
	Divorced	3 (0.8)
Education	Illiterate	108 (28.1)
	Less than 8 years of education	242 (62.9)
	More than 8 years of education	25 (6.5)
	College	10 (2.5)
Profession	Retired	175 (43.5)
	Housewife	205 (53.2)
	Worker	3 (0.8)
	Officer	0 (80)
	Business owner	1 (0.3)
	Other	1 (0.3)

pneumococcal, and tetanus) were deficient in 98.4%, 96%, and 94.5% of the patients, respectively (Table 3).

There were no significant differences in PIMs and PPOs between the sexes ($p>0.05$). There were no significant differences in PIMs and PPOs between patients aged 65-74 years and those aged over 75 years ($p>0.05$).

When evaluated according to educational status, there was no statistically significant difference in terms of inappropriate drug use according to the TIME-to-STOP and TIME-to-START criteria in the participants who were illiterate, those with less than eight years of education, those with more than eight years of education, and those who had graduated from college.

Concerning the chronic disease distribution of the volunteers participating in the study, the following results were obtained: 1.6% (never), 16.6% (one), 35.8% (two), 29.9% (three), 11.4% (four), 3%.9 (five), 0.5% (six), 0.3% (seven). The most common chronic diseases in our study were hypertension, diabetes mellitus, and dyslipidemia. There was no significant difference in the number of diseases between patients with and without PPO who were evaluated for PPOs according to the TIME-to-START criteria. However, according to the TIME-to-STOP criteria, the number of diseases in patients with PIMs was found to be significantly higher than that in patients without PIMs ($p<0.05$).

Table 2. Potentially Inappropriate Medication according to TIME to-STOP criteria	
	(%) (n=385)
A2. The use of digoxin in doses higher than 0.125 mg/day is not appropriate (risk of toxicity).	1% (4)
A3. The use of digoxin is not appropriate in the indication of heart failure with preserved (normal) ejection fraction.	0.5% (2)
A6. Loop diuretic use is not appropriate for ankle edema without signs of heart failure, liver failure, nephrotic syndrome, or renal failure.	1.8% (7)
A8. In patients with urinary incontinence, the use of diuretics as the first step is not appropriate for the treatment of essential hypertension (it may impair quality of life by increasing incontinence and feeling of urgency, may increase falls).	1% (4)
A9. The use of alpha-1 blockers or centrally acting antihypertensives (e.g. methyldopa, rilmenidine, reserpine) is not appropriate in the treatment of hypertension, except when other classes of antihypertensives are not tolerated or are ineffective (increased heart failure and cardiovascular events, orthostatic hypotension, decrease with alpha-1 blocker antihypertensives), syncope, worsening of urinary incontinence in women; central nervous system side effects of centrally acting antihypertensives, sedation-depression-parkinsonism and orthostatic hypotension, bradycardia side effects).	1.3% (5)
A10. Because of the increased risk of orthostatic hypotension, vasodilator antihypertensive agents and nitrates are not suitable for use in patients with orthostatic hypotension.	0.8% (3)
A11. Tight blood pressure control (<140/90 mmHg) is not appropriate in patients with orthostatic hypotension/cognitive impairment (e.g. dementia)/functional limitation/low life expectancy (<2 years)/high risk of falling.	3.1% (12)
A16. Potassium-sparing drugs (aldosterone antagonists, triamterene, amiloride, ACEI, ARB) are not suitable for use in patients with GFR <30 mL/minute/1.73 m^2 and whose serum potassium level cannot be closely monitored (risk of hyperkalemia).	1.8% (7)
A18. NSAID use is not appropriate in patients with cardiovascular disease (severe hypertension, heart failure or previous myocardial infarction, stroke) (increased cardiovascular event: risk of myocardial infarction, stroke, heart failure and death).	1.3% (5)
A19. Beta-blocker use is not appropriate in diabetes mellitus patients with frequent hypoglycemic episodes.	1.3% (5)
A21. Long-term use of aspirin at doses higher than 75-150 mg/day is not appropriate for cardiovascular protection (both primary and secondary) (no proven additional benefit and increases bleeding risk).	0.3% (1)
A23. Concomitant use of aspirin and clopidogrel for secondary stroke prophylaxis is not appropriate unless there is a specific indication for concomitant use.	0.3% (1)
A30. The use of drugs with a narrow therapeutic index, such as warfarin and digoxin, is not appropriate for patients who have difficulty in using and managing their medication (e.g. patients with cognitive impairment) and who are not available to assist (e.g. caregivers) (risk of life-threatening toxicity).	0.3% (1)
B1. Tricyclic antidepressant use is inappropriate (high anticholinergic effect, cognitive deterioration, cardiac conduction disorder, orthostatic hypotension, urinary retention, worsening of prostatism, worsening of narrow-angle glaucoma).	0.3% (1)
B6. In case of GFR <60 mL/minute/1.73 m^2, the use of pregabalin and gabapentin without dose reduction is not appropriate.	1.6% (6)

Table 2. continued

	(%) (n=385)
B14. Benzodiazepines are not suitable for use for more than 4 weeks (prolonged sedation, confusion, balance disorder, falling, risk of traffic accidents).	0.3% (1)
B18. Continuous and long-term use of drugs such as betahistine, trimetazidine, dimenhydrinate is not appropriate in the treatment of vertigo (lack of evidence-based beneficial effects).	0.5% (2)
B20. Piracetam is not suitable for use except in the treatment of myoclonic convulsions.	0.8% (3)
B21. First-line use of carbamazepine, phenytoin, phenobarbital, or valproate is not appropriate in the chronic treatment of epilepsy (due to adverse effects on vitamin D, enzyme induction, risk of falling; there are also safer alternatives).	0.5% (2)
B22. Tramadol, neuroleptics/antipsychotics (clozapine, olanzapine, chlorpromazine, thioridazine), bupropion and maprotiline are not suitable for epilepsy patients.	0.3% (1)
B24. The use of citalopram at doses above 20 mg/day and escitalopram over 10 mg/day in the older adultsis not appropriate (due to the risk of QTc prolongation).	0.5% (2)
C1. Concomitant use of non-steroidal anti-inflammatory drugs with oral anticoagulants (vitamin K antagonists, direct thrombin inhibitors, factor Xa inhibitors) is not appropriate (risk of gastrointestinal bleeding).	1% (4)
C2. Aspirin, clopidogrel, non-steroidal anti-inflammatory drugs or steroids; It is not suitable for use without proton pump inhibitor in patients with a history of ulcer, in patients receiving additional antiplatelet therapy, in patients receiving concomitant anticoagulants, in patients using steroids, and in patients with dyspepsia-GER symptoms.	9.6% (37)
C3. Aspirin or non-steroidal anti-inflammatory drugs; Patients with a history of peptic ulcer (complicated or uncomplicated, gastric, or duodenal) are not suitable for chronic use without testing for *Helicobacter pylori*.	0.3% (1)
C4. For the treatment of erosive peptic esophagitis or uncomplicated peptic ulcer, the use of proton pump inhibitors at a therapeutic dose for longer than 8-12 weeks is not appropriate. (dose reduction or shorter interruption is indicated).	0.8% (3)
C5. Proton pump inhibitor use is not appropriate due to multiple drug use (no benefit, potential harm).	2.1% (8)
C6. The use of anticholinergic gastrointestinal antispasmodics (e.g. hyoscyamine) is not appropriate. Increased anticholinergic side effects (dizziness, decreased cognitive abilities, blurred vision, arrhythmia, bloating-constipation) and limited utility in theolder adults.	1% (4)
C7. In patients with chronic constipation, if there are alternatives that do not have this side effect, the use of drugs with a high probability of causing constipation (drugs with high anticholinergic effects, oral iron, opioids, verapamil, aluminum antacids) is not appropriate (risk of increased constipation).	1.3% (5)
D1. The use of antimuscarinic bronchodilator drugs (ipratropium, tiotropium) is not appropriate in patients with narrow-angle glaucoma or urinary outflow obstruction (risk of worsening glaucoma and urinary retention).	0.5% (2)
D2. Theophylline is not suitable for the maintenance treatment of COPD or asthma bronchialene (due to the narrow therapeutic index and high risk of insomnia, arrhythmia in theolder adults).	2.1% (8)
E1. If there is an additional alternative treatment, it is not appropriate to use non-steroidal anti-inflammatory drugs for more than 3 months.	0.5% (2)
E2. Non-steroidal anti-inflammatory drugs are not suitable for use in patients with GFR <50 mL/minute/1.73 m^2 (risk of worsening renal function).	0.5% (2)
E11. The use of systemic muscle relaxant (skeletal muscle) agents (thiocolchicoside, tizanidine, chlorzoxazone, carisoprodol, chlorphenezine carbamate, cyclobenzaprine, metaxalone, methocarbamol and orphenadrine ... etc.) is not suitable for musculoskeletal pain (sedation, drowsiness, dizziness, dry mouth), constipation, due to cognitive side effects).	0.5% (2)
F3. Anticholinergic drug use for the bladder is not appropriate without post micturition residue determination (risk of urinary retention and postrenal kidney failure) in the older adults with prostate hyperplasia (obstruction risk) or diabetes mellitus complications (neurogenous bladder risk) or frail older adults (reduced contractility risk with detrusor hyperactivity).	0.5% (2)
F5. Non-uroselective alpha 1 blockers (e.g. doxazosin, terazosin) are not suitable for the treatment of lower urinary tract symptoms due to benign prostatic hyperplasia in patients with orthostatic hypotension (increased orthostatic hypotension, syncope, and falls).	0.3% (1)
G1. Tight blood glucose control (HbA1C <7%) is not appropriate in patients with a low life expectancy (<5 years) or a history of decline or cognitive impairment.	2.3% (9)
G2. The use of metformin is not appropriate in the frail or malnourished older adults (due to the gastrointestinal side effects and anorexia effect of metformin).	0.8% (3)
G5. The use of thiazolidinediones (rosiglitazone, pioglitazone) is not appropriate in patients with documented heart failure/fracture history/ increased fracture risk/bladder cancer history or on insulin therapy (worsening heart failure, increased fracture, and bladder cancer risk).	0.3% (1)
G8. It is not appropriate to use SGLT-2 inhibitors in cases with GFR <45 mL/minute/1.73 m^2.	0.5% (2)
H1. Use of drugs with high anticholinergic effects in conditions such as falls, dementia, constipation, narrow-angle glaucoma, delirium, urinary retention, concomitant use of drugs with high anticholinergic effects not suitable.	0.5% (2)

ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, SGLT-2 inhibitors: Sodium glucose Co-transporter 2 inhibitor, COPD: Chronic obstructive pulmonary disease, GER: Gastroesophageal reflux, HbA1C: Hemoglobin A1C, GFR: Glomerular filtration rate, NSAID: Non-steroidal anti-inflammatory drugs

Table 3. Potential prescribing omissions according to TIME to-START criteria

	(%) (n=385)
A1. It is appropriate to initiate antiplatelet therapy (aspirin or clopidogrel) for secondary prevention in patients with atherosclerotic coronary artery disease and a history of atherosclerotic cerebrovascular disease.	0.8% (3)
A2. Atherosclerotic coronary artery disease, cerebrovascular disease or statin for secondary prevention in patients with peripheral artery disease It is appropriate to start treatment.	2.1% (8)
C1. In cases with symptomatic constipation unresponsive to lifestyle changes (diet-exercise), it is appropriate to exclude fecal plug and start fiber support (psyllium, methylcellulose, polycarbophil, wheat dextrin) or polyethylene glycol.	45.5% (175)
E1. It is appropriate to initiate replacement therapy in patients with daily dietary vitamin D intake <800-1000 IU or elemental calcium intake <1000-1200 mg.	0.5% (2)
E2. It is appropriate to initiate an anti-resorptive (bisphosphonate, denosumab) or anabolic agent (parathormone analog) in patients with documented osteoporosis with fragility fracture and/or bone mineral densitometry T-score (femur total, femoral neck or lumbar <-2.5).	0.5% (2)
E8. It is appropriate to start a xanthine oxidase inhibitor (primarily allopurinol) in patients with recurrent gout attacks.	0.3% (1)
H1. Annual influenza vaccination is appropriate.	74.8% (288)
H2. Pneumococcal vaccine (one dose for each of the 13 valent conjugate and 23 valent polysaccharide vaccines) is appropriate.	96.9% (373)
H3. Herpes zoster vaccination is appropriate (reduces the risk of shingles infection and postherpetic neuralgia).	98.4% (379)
H4. It is appropriate to perform tetanus vaccine (tetanus-diphtheria toxoid) every 10 years.	94.5% (364)
I1. In the older adults with malnutrition or malnutrition risk, initiation of oral nutritional supplements is appropriate if nutritional counseling and nutritional supplementation are not sufficient to increase dietary intake and achieve nutritional goals.	3.4% (13)

Discussion

Country-specific criteria are more effective for PIMs and PPOs. As shown in the first publication of our study, country-specific criteria for detecting PIMs and PPOs were found to be significantly superior (6). Since our study is one of the first conducted studies with the TIME criteria, it can have an important place in terms of providing resources and comparison opportunities for studies to be conducted in Turkey for IMU. In particular, we shared the distribution of PIMs and PPOs in detail.

In addition, differences are detected when examining the distribution of PIMs and PPOs; in particular, country-specific prescribing habits affect this. Even when compared with comprehensive studies such as Optimizing Therapy to Prevent Avoidable Hospital Admissions in Multimorbid Older Adults, PIMs and PPOs are concentrated on different criteria (7). According to the results of a study conducted with 206 patients using STOPP v2, the distribution of PIMs was related to dietary supplements such as vitamins and minerals (22.1%), antihypertensive drugs (17.2%), and PPIs (10.7%) (8). In our study, only the inappropriate use of PPIs was similar in the first 3 PIMs. When the studies conducted using the STOPP/START v1 and v2 in China were examined, the most determined criteria still differed (9).

Considering the study conducted in Turkey comparing Beers 2012 and the STOPP version 2, the rates of PIM use in Turkey were found to be 33.3% and 39.1%, respectively. In our study, it was 33% according to TIME criteria. Although the rate in our study seems to be lower when comparing the rates of PIM use, this may be because the two studies were conducted in different years. This reflects that recent studies on IMUs have reached their goal. When the most common drugs related to PIMs were examined, PPIs came to the fore in our study, whereas Bahat et al. (10) found that aspirin and antipsychotics were prominent in their study. Our study could not objectively evaluate geriatric syndromes such as dementia, frailty, and malnutrition. Therefore, the main reason for the differences between the most commonly used inappropriate drugs may be the difference in the evaluation of patients rather than the change in prescribing habits. We think that other reasons may be due to the change in the habits of prescribing according to years or the studies examining patients from two different outpatient clinics, such as geriatrics and internal medicine.

In addition, in countries where the official language is not English and the rate of foreign language proficiency is low, the existence of country-specific criteria for combating PIMs and PPOs is crucial. Therefore, the success rate of the TIME criteria increases (11). To some extent, this complicates comparison studies with popular medication lists such as the Beers criteria and START/STOPP. International validity studies of TIME criteria based on the Delphi process were also conducted. According to the results of this study, the criteria set can be applied in both central and eastern Europe (12).

An increase in the use of various mobile applications in the near future can be beneficial for IMUs. Simultaneously, clinicians trained on this subject and increasing cooperation with clinical pharmacology disciplines can decrease the rates of PIMs and PPOs (3).

As a result of the increase in the older adult population in Turkey, measures are taken and studies are carried out by the official authority on this issue. The rate of population increase of those aged 65 years and over in Turkey was 3.3% in 1950, 6.7% in 2000, and 9.5% in 2020, with a population that has reached 7,953,555. It is predicted that this rate will increase to 10.2%, 16.3%, and 25.6% in 2023, 2040, and 2080, respectively (13). According to the latest TUIK data, the population aged 65 and over was 8 million 451 thousand 669 people in 2022, and its ratio in the total population increased to 9.9% in 2022.

Expanding the use of the TIME criteria, which is a criterion specific to Turkey, and supporting it with mobile applications should be among the targets that need to be urgently addressed in this regard (14).

There are three different terms in the World Health Organization [(WHO); Adherence to long-term therapies: Evidence for action] report: "compliance, adherence, concordance". Compliance is defined as the patient's use of the drug at the given dose at the recommended intervals for the required time according to the treatment protocol. It is necessary to increase health literacy to improve this situation, which is very important for preventing IMU (15).

Health literacy is when a patient is given medical information, understands, and interprets this information, and acts accordingly. Although health literacy was first defined by Simond in 1974, its importance has only recently been understood in Turkey. As a result of the "Reliability and Validity Study of the Turkish Health Literacy Scales (TSOY-32)" conducted in 2016 under the editorship of Abacigil et al. (16) with the contribution of the Ministry of Health, the Turkish Adaptation of the European Health Literacy Scale and the TSOY-32 for Turkish society were added to the literature. In Hazer and Ateşoğlu (17) in the mainland province of Turkey, the health literacy of older adult individuals was insufficient and problematic. The success of healthy aging in older adults with high health literacy was also high (17). The importance of health literacy in maintaining and improving the level of health on both an individual and community basis has been emphasized by the WHO (18).

In a study using STOPP/START version 2, PIMs were 62.5% and PPOs were 36.6% (not including vaccine data). Including vaccine data, 225 (97%) patients had at least one PPO (19). The inclusion of vaccine data significantly increased the rate of PPOs, as also observed in our study. This demonstrates the importance of immunization for PPOs and how they fall behind. In another study conducted in Australia, when vaccination rates were included, PPOs (99%) were similarly high as in our study (20). In a study conducted in the Netherlands, PPOs was 84.8% (ranging from 77.4 to 90.6%) (21). In PPOs, vaccination deficiencies were the most common. Physicians should be more conscious of adult immunization needs when providing healthcare to the older adult population.

Consequently, both PIMs and PPOs are gaining importance in older individuals. This is one of the main factors in the prevention of IMU, polypharmacy, ADRs and ADEs. The TIME criteria better reveal PIMs and PPOs in Turkish society.

Study Limitations

There are some limitations to this study. The first is that it is a single-center study. It also has a modest sample size. Studies in the literature with IMUs are generally multicentered and performed with large patient groups. Another limitation of the study is that some TIME criteria related to malnutrition, frailty, and dementia cannot be evaluated objectively.

Conclusion

Our study is one of the first IMU studies conducted with TIME criteria and reveals important findings about the prescribing habits of Turkey. Turkey will be an important resource for the comparison of national and international criteria for the prevention of IMU. Extending the use of country-specific criteria in clinical practice should be one of our important goals.

Ethics Committee Approval: Ethics committee approval of our study was given by the Kütahya Health Sciences University (Turkey) Non-Invasive Clinical Research Ethics Committee (approval nubmer: 2020/04-15, date: 25.02.2020).

Informed consent: Informed consent forms were obtained from all patients.

Peer review: Externally peer-reviewed.

Authorship Contributions: Concept - E.B., T.P.K., F.Ö.; Design - E.B., T.P.K., F.Ö.; Data Collection or Processing - E.B., T.P.K.; Analysis or Interpretation - E.B., T.P.K.; Literature Search - E.B., T.P.K., F.Ö.; Writing - E.B., T.P.K.

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Differentiating Pulmonary Tuberculosis from Bacterial Pneumonia: The Role of Inflammatory and Other Biomarkers

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ABSTRACT

Introduction: To investigate the values of procalcitonin (PCT) and laboratory parameters in differentiating tuberculosis (TB) pneumonia from community-acquired pneumonia (CAP).

Methods: Between 01.01.2018 and 01.01.2020, 4,133 patients diagnosed with CAP or TB pneumonia in a reference hospital for chest diseases and TB were retrospectively screened. Patients with a history of close contact with someone with TB, night sweats, weight loss, or cavitary infiltration that may be typical for TB in the chest X-ray were evaluated clinically and radiologically as high TB suspects and were excluded from the study. Demographic characteristics, comorbidities, medications, admission complaints, radiological findings, microbiology results, and laboratory parameters were recorded. CURB-65 and pneumonia severity index scores were calculated. Patients were grouped as TB (n=70) and CAP (n=506) based on the final diagnosis. The parameters of the two groups were compared.

Results: The mean age of 576 patients was 55, and 423 (73%) were male. While sodium (Na) [95% confidence interval (CI): 0.716-0.914, p=0.001], blood urea nitrogen (BUN) (95% CI: 0.910-0.986, p=0.008), alanine aminotransferase (ALT) (95% CI: 0.913-0.998, p=0.043) and oxygen saturation (95% CI: 1.007-1.268, p=0.037) were found as independent biomarkers for differentiating TB and CAP, PCT had no significant influence on the differential diagnosis.

Conclusion: Prompt differential diagnosis between TB and CAP is important for public health in endemic TB areas. We recommend the evaluation of Na, ALT, and BUN for this purpose.

Keywords: Biomarkers, tuberculosis pneumonia, procalcitonin, community-acquired pneumonia, hyponatremia

Introduction

Community-acquired pneumonia (CAP) is a severe disease frequently encountered despite the use of many effective antibiotics and vaccines. However, tuberculosis (TB) continues to be a public health problem, the dimensions of which are increasing in our country and many developing countries, as it is in most of the world's geography (1).

CAP is one of the most common infections that cause death in adults and is the main reason for hospitalizations (2). Prompt diagnosis and early appropriate antibiotic therapy are essential to reduce morbidity and mortality from CAP. In countries where TB is common, *Mycobacterium tuberculosis* is a common cause of CAP (3-5). Differential diagnosis of TB from CAP is difficult because of the clinical and radiographic findings that vary according to the age and comorbidity of the patient and the low sensitivity of acid-fast bacillus microscopy (6,7). Therefore, the additional diagnostic method that can be used to differentiate between the two diseases will be of clinical importance in terms of isolating patients with TB and initiating appropriate anti-TB drug therapy at an early stage.

Diagnostic methods and early indicators of the extent of the inflammatory response can help in our initial treatment decisions.

Patients admitted to hospitals with CAP are often treated empirically for multiple organisms while awaiting research results. This costly approach may develop antibiotic resistance while exposing patients to potentially harmful pharmacotherapy or overdosage. Ultimately, it can lead to inappropriate treatment, negatively affecting morbidity and mortality. At the same time, even if the current diagnosis is TB, empirical antibiotic therapy facilitates the mechanisms that lead to the development of resistance to the major drugs used in treating TB.

C-reactive protein (CRP) is an acute phase protein and a non-specific marker of systemic inflammation (8). Procalcitonin (PCT) is a 116 amino acid protein with the same sequence as the prohormone calcitonin.

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It is synthesized by leukocytes and is found in high concentrations in the blood in bacterial infections and inflammatory response syndrome (9,10).

TB is associated with high circulating inflammatory cytokine levels, especially tumor necrosis factor-alpha (11,12). This cytokine has been shown to induce PCT production independent of the presence of bacterial endotoxins (13) and may explain the high PCT levels found in conditions other than bacterial sepsis, such as malaria (14) and systemic fungal infections (15).

Studies on the role of serum CRP concentration in determining the etiology of CAP and predicting its prognosis have been conducted (16-18), and it has been found that PCT is a better alternative to CRP in guiding antibiotic therapy in acute exacerbations of CAP and COPD (19,20). This result was attributed to the ability to distinguish between patients with and without bacterial infections. On the other hand, it was observed that PCT did not significantly increase in patients with pulmonary tuberculosis (PTB) (21,22). It can be concluded that it could be used as an attractive rapid differential diagnosis method to differentiate PTB from bacterial CAP.

PTB and CAP are common causes of lower respiratory tract infections and may have similar clinical and radiological features. It is important to accelerate the diagnosis and treatment planning in highly contagious disease groups such as TB pneumonia and to start isolation and specific treatment early to protect public health. For this reason, it is important to guide the physicians of other branches working in primary and secondary healthcare centers in differential diagnosis and treatment management.

The primary aim of this study was to investigate the diagnostic value of serum PCT and CRP sedimentation levels, which are inflammatory markers, to differentiate TB pneumonia in patients evaluated with the diagnosis of pneumonia. Its secondary purpose is to evaluate the auxiliary role of other laboratory markers and findings in diagnosing TB. The objective of the study was to facilitate the differential diagnosis between two common diseases to accelerate the diagnosis and consider microbiologic samples and initiating isolation for TB at the earliest to protect public health.

Methods

The study is a single-center and retrospective study that was conducted after being approved by the University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital Scientific Board and Ethics Committee (approval number: 2021-102, date: 25.03.2021).

From the Hospital Information Management System between 01.01.2018 and 01.01.2020, 4,133 patients diagnosed with bacterial pneumonia and TB pneumonia by our hospital chest diseases clinics and TB clinic were identified. The files of all patients were re-evaluated, and patients under the age of 18, non-infective pneumonia cases, human immunodeficiency virus (+) patients, patients with high clinical suspicion (cough, night sweats, weight loss, close contact with someone with active TB) and radiological typical TB appearance (presence of cavitary infiltration in localizations that may coincide with lung areas where TB is common, such as upper lobe posterior and lower lobe superior), pregnant patients, patients who had undergone trauma or recent surgery, those with a lung cancer diagnosis, and additional immunosuppressive disease, patients with systemic inflammatory comorbidity, bronchiectasis patients, and multidrug-resistant (MDR) TB patients were excluded. A total of 576 patients (506 CAP, 70 TB) with all available baseline data recorded for the study were included (Figure 1).

Final diagnostic criteria: presence of clinical complaints such as cough and sputum with pneumonic infiltration without cavitary appearance in the chest X-ray, meeting the criteria for inclusion in the study, and monitoring clinical and radiological response after the treatment started.

TB diagnostic criteria: Presence of radiological lobar, multilobar or bronchopneumonic involvement and presence of acid-fast bacilli in sputum or bronchoalveolar lavage fluid, provided that cases with high clinical suspicion of TB. and radiological typical cavitary lesions are excluded.

CAP diagnostic criteria: Presence of radiological lobar, multilobar, or bronchopneumonic involvement in the presence of appropriate symptoms and physical examination findings, absence of acid-fast bacilli in the tests performed for the detection of the causative microorganism.

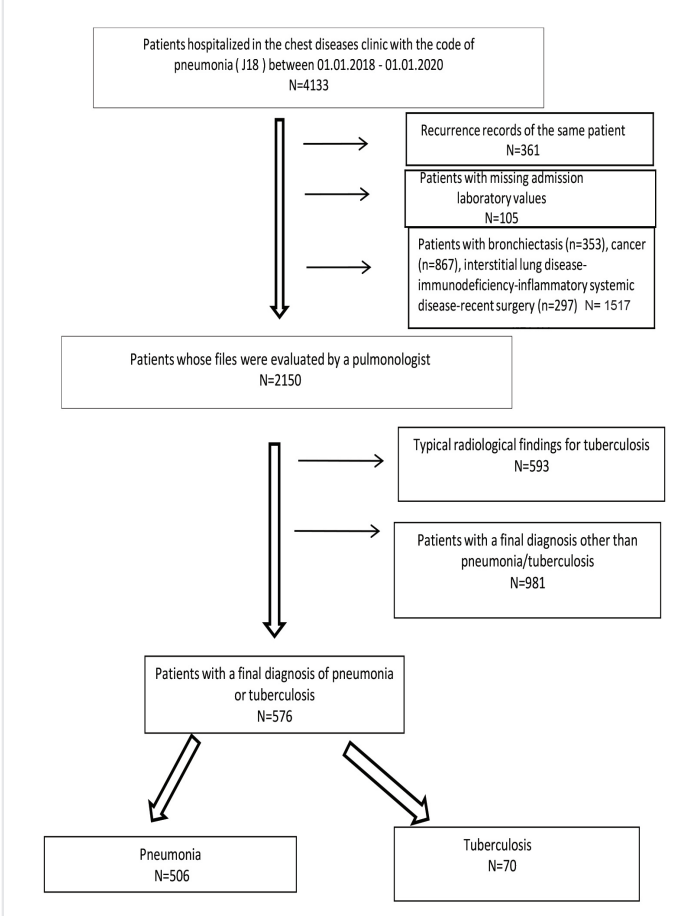

Figure 1. Flowchart for patient inclusion

Recorded Data

Patient age, gender, comorbidities, medications, admission complaints, radiological findings, basal hemogram, biochemistry data, arterial blood gas values, microbiological culture results, and direct examination results were recorded. Drug resistance patterns of TB patients were investigated. The CURB-65 score and pneumonia severity index (PSI) were calculated. Among the biochemistry data, urea, creatinine, PCT, CRP, aspartate aminotransferase, alanine aminotransferase (ALT), sodium (Na), potassium, chlorine (Cl), alkaline phosphatase, bilirubin, gamma glutamyl transferase, lactate dehydrogenase (LDH), and uric acid levels were recorded.

Statistical Analysis

Quantitative data are expressed as mean ± standard deviation and qualitative data are expressed as frequencies. Chi-square and Student's t-tests were used to evaluate the data obtained from the intergroup comparison. Variables found significant in univariate analysis were then incorporated into multiple logistic regression analysis. The cut-off value was determined for markers with $p<0.01$ in multi-way analyzes. The cut-off values for Na and blood urea nitrogen (BUN) levels were obtained from receiver operating characteristic (ROC) curves. All statistical analyzes were carried out using a statistical software package system (SPSS for Windows, version 16.0; SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

Results

The mean age of all 576 patients was 55, and 423 (73%) were male. The most common complaints at admission were dyspnea (n=430, 75%) and cough (n=300, 52%), while 6 patients were asymptomatic. The final diagnosis was TB in 70 (12%) patients and pneumonia in 506 (88%) patients.

While smear positivity in 48 (69%), mycobacteria PCR positivity in 48 (69%), and culture positivity in 65 patients were recorded in PTB patients, the diagnosis was made hytopathologically in 5 patients. At least one non-MDR drug resistance, most commonly isoniazid, was recorded in 11 patients (Table 1).

When PTB and CAP patients were compared, it was seen that PTB patients were younger (51±20 vs. 65±15, $p<0.0001$) and the frequency of comorbid disease was lower (43% vs. 71%, $p<0.0001$). In CAP patients, dyspnea, and sputum were more common, and fatigue and weight loss were less frequent.

Among the additional diseases, heart failure (15% vs. 6%, $p=0.041$), renal failure (9% vs. 2%, $p=0.047$), and chronic obstructive pulmonary disease (COPD) (46% vs. 20%, $p<0.0001$) were more frequent in CAP patients. The frequency of TB history or index cases was less common in CAP patients (%14 vs. %23, $p<0.0001$).

Among the vital signs, tachypnea was detected more frequently in CAP (42% vs. 24%, $p=0.005$). CURB-65 and PSI scores were higher in CAP patients.

In laboratory findings in one-way analysis, PTB patients had lower leukocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), higher platelet (PLT) (pneumonia 283 TBC 338), lower platelet distribution width (PDW) lower, neutrophil (Ne) lower, eosinophils (Eo) higher, CRP lower, BUN lower, Na, ALT, and LDH lower, saturation was higher and statistically significant compared to CAP patients (Table 1).

WBC, MCV, Ne, PLT, CRP, Eo, BUN, LDH, Na, ALT, and saturation values, which were found to be significant between the two groups, were included in the logistic regression analysis. Accordingly, among the laboratory parameters, Na [odds ratio (OR): 0.809, 95% confidence interval (CI): 0.716-0.914, $p=0.001$], BUN [OR: 0.947, 95% CI: 0.910-0.986, $p=0.008$, ALT (OR: 0.955, %) 95 CI: 0.913-0.998, $p=0.043$] and oxygen saturation (OR: 1.133, 95% CI: 1.007-1.268, $p=0.037$) were found to be independent markers in the differentiation of PTB and CAP (Table 2).

Na and BUN were found to be predictors of PTB in ROC analysis. The predictor value for Na is 137 [area under the curve (AUC): 0.627: $p=0.001$, 56% sensitivity and 55% specificity] (Figure 2).

The preditor value for BUN is 37 (AUC: 0.760, $p<0.0001$, 75% sensitivity and 72% specificity) (Figure 3).

Discussion

In our study, when laboratory findings of PTB patients were compared with CAP patients, leukocytes, MCV, and MCH were lower, PDW was lower, Ne lower, CRP lower, BUN lower, Na, ALT, and LDH lower, PLT and Eo were higher, and saturation was higher and found to be significant compared to CAP patients. In multivariate analyzes baseline Na, BUN, ALT, and saturation values were independently significant for the two diseases.

PTB patients were younger and had fewer comorbidities. COPD was the most common comorbidity, followed by cardiovascular diseases. Since the CAP patients were older, COPD was observed more frequently as a comorbid disease, and these patients had more dyspnea, sputum complaints, and more deterioration in vital values such as hypotension, tachypnea, and low oxygen saturation. These patients had no history of TB or close contact with someone with active TB. In addition to the clinical and radiological evaluation, adequate medical history and investigation of risk factors are also important in determining the differential diagnosis and empirical treatment.

Pneumonia produces an inflammatory response that releases acute phase proteins. The fact that erythrocyte sedimentation rate (ESR) and leukocyte count, which are used to measure this response, can be affected by many infectious and inflammatory factors, reducing their sensitivity, and many studies on the acute phase response have shown that CRP levels have a superior diagnostic efficiency in determining bacterial pneumonia in adults (23). Our study found no significant difference in ESR levels; leukocyte and CRP levels were lower in PTB patients.

When PCT values were examined, no distinctive statistically significant difference was found between the two diseases. Although it was stated in the current studies that low PCT was a predictor in favor of TB in the differentiation of TB pneumonia and CAP, no distinctive statistically significant difference was found between the two diseases in our study.

Table 1. Comparison of recorded clinical findings according to the final diagnosis

	All patients	Pneumonia	TBC	p-value
	(n=576)	(n=506)	(n=70)	
Gender (n, %)				
Male	423 (73)	373 (74)	50 (71)	0.667
Female	153 (27)	133 (26)	20 (29)	
Mean age; (years ± SD, minimum-maximum)	63.1±16.6 (17-100)	65±15	51±20	<0.0001
Admission complaints (n, %)				
Shortness of breath	430 (75)	397 (79)	33 (47)	<0.0001
Cough	300 (52)	261 (52)	39 (56)	0.527
Sputum	193 (35)	187 (37)	17 (24)	0.045
Temperature	155 (27)	142 (28)	13 (19)	0.113
Weakness	67 (12)	51 (10)	16 (23)	0.004
Hemoptysis	47 (8)	37 (7)	10 (14)	0.06
Pain (back/chest)	56 (10)	50 (10)	6 (10)	0.833
Weight loss	14 (2)	8 (2)	6 (9)	0.004
Other	53 (9)	42 (9)	8 (14)	0.068
Asymptomatic	6 (2)	2	4	0.054
Presence of additional disease (n, %)	388 (67)	358 (71)	30 (43)	<0.0001
Additional diseases (n, %)				
IHD	123 (21)	109 (22)	14 (20)	0.877
CHF	80 (14)	78 (15)	4 (6)	0.041
CKD/AKF	47 (8)	46 (9)	1 (2)	0.032
Liver disease	4 (1)	4	0	0.998
CVA	9 (2)	8 (2)	1 (2)	0.998
DM	125 (22)	112 (22)	13 (19)	0.541
COPD	246 (43)	232 (46)	14 (20)	<0.0001
Hypertension	46 (8)	44 (9)	2 (3)	0.102
Asthma	8 (1)	8 (2)	0	0.607
TBC history (n, %)	84 (15)	68 (14)	16 (23)	<0.0001
Antibiotics use before admission (n, %)	266 (48)	261 (53)	30 (48)	0.590
Vital values (n, %)				
Temperature (<35/>40)	122 (22)	113 (23)	9 (13)	0.112
HR >125	121 (21)	105 (21)	17 (25)	0.429
RR >30	225 (40)	209 (42)	16 (24)	0.005
BP >90	22 (4)	19 (4)	3 (5)	0.736
CURB-65 mean score	1.9±0.9	1.9±0.8	1.3±0.9	<0.0001
CURB-65 score (n, %)				
0	33 (6)	22 (4)	11 (6)	-
1	152 (26)	121 (24)	32 (47)	-
2	254 (44)	235 (47)	19 (28)	<0.0001
3	125 (22)	118 (24)	7 (10)	-
4	8 (1)	8 (2)	0	-
PSI group (n, %)				
0	2 (0.3)	2 (0.4)	0	-
1	93 (16)	65 (13)	29 (41)	-
2	89 (16)	76 (15)	13 (20)	<0.0001
3	120 (21)	111 (22)	10 (15)	-
4	207 (36)	193 (38)	14 (20)	-
5	61 (11)	57 (11)	4 (7)	-

SD: Standard deviation, BP: Blood pressure, CHF: Congestive heart failure, CKD/AKF: Chronic kidney disease/acute kidney injury, COPD: Chronic obstructive pulmonary disease, CRP: C-reactive protein, CVA: Cerebrovascular accident, DM: Diabetes mellitus, HR: Heart rate, IHD: Ischemic heart disease, PSI: Pneumonia severity index, RR: Respiratory rate

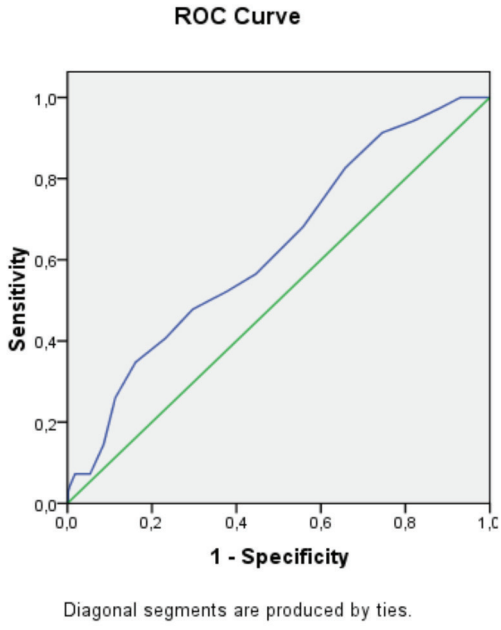

Figure 2. ROC curve for sodium
ROC: Receiver operating characteristic

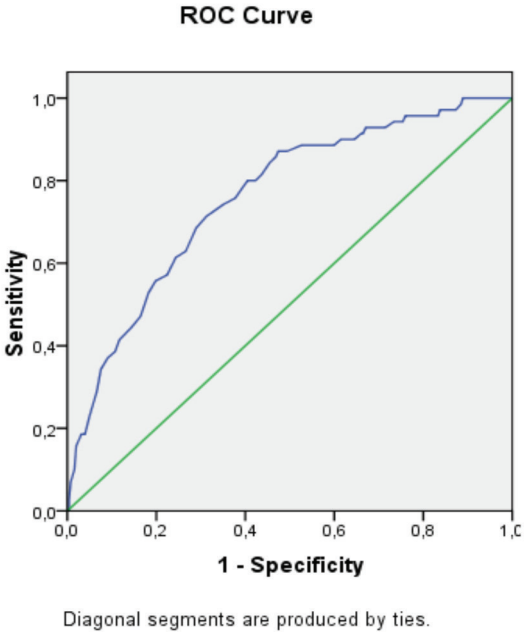

Figure 3. ROC curve for blood urea nitrogen levels (BUN)
ROC: Receiver operating characteristic, BUN: Blood urea nitrogen

This current finding may be due to the significant difference between our sample sizes and atypical bacterial agents and viral infections that do not cause a significant increase in PCT. In our study, the responsible bacterial or viral agents in each infection could not be determined, and it was determined that there were not sufficient tests for the isolation of the agent in some data. At the same time, since our hospital is a tertiary education and research hospital, the fact that the patients spent the first

Table 2. Multiple logistic regression analysis according to the final diagnosis

	OR	95% CI	p-value
WBC	1.009	0.702-1.452	0.960
MCV	0.979	0.895-1.071	0.647
Ne	0.909	0.609-1.358	0.642
PLT	1.004	0.999-1.009	0.138
CRP	0.998	0.990-1.005	0.518
Eo	1.603	0.126-10.419	0.716
BUN	0.947	0.910-0.986	**0.008**
LDH	1.005	0.999-1.011	0.120
Na	0.809	0.716-0.914	**0.001**
ALT	0.955	0.913-0.998	**0.043**
Saturation	1.133	1.007-1.268	**0.037**

ALT: Alanine transaminase, BUN: Blood urea nitrogen, CRP: C-reactive protein, Eo: Eosinophil, LDH: Lactate dehydrogenase, MCV: Mean corpuscular volume, Na: Sodium, Ne: Neutrophil, PLT: Platelet, WBC: White blood cell, CI: Confidence interval, OR: Odds ratio

24-48 hours from the onset of symptoms until they applied to us may also have a role in this.

A comparative study by Quist and Hill (24) on TB, bacterial, and Pneumocystis carinii pneumonias found that LDH levels were high in 73% of bacterial pneumonias, and AST and ALT levels were high in 74%.

It has been reported that elevated LDH, BUN, and ALT levels are associated with poor prognosis and mortality in CAPs (25). In our study, where ALT and LDH levels were significantly lower in TB pneumonia patients, we can also attribute this to the fact that our CAP patients were older patients with comorbid diseases.

Hyponatremia, an electrolyte disorder, is relatively common in patients presenting with pneumonia and has been associated with disease severity. The exact mechanism is not fully understood, but inappropriate antidiuretic hormone (ADH) secretion is thought to play a role in the etiology, and low serum Na level has been associated with a poor prognosis (26-29). Zilberberg et al. (27) found that pneumonia patients with hyponatremia had more intensive care and extended hospital stays.

Factors such as interleukin-6, one of the inflammatory cytokines, stress, hypoxia, and nausea are associated with the non-nosmotic stimulus of ADH (30). In addition to pneumonia, ADH stimulation can be seen in infections such as TB and malaria.

The low BUN, which usually occurs due to not meeting the amount of protein needed by the body with nutrition, may also be due to excessive fluid consumption. In addition to the low socioeconomic level in TB patients, anorexia, and malnutrition that develops with the chronic course of the disease, the anabolic process in which the body enters may explain the low BUN, which is more pronounced in TB than in pneumonia.

In countries where TB is common, it is important to distinguish between TB pneumonia and CAP, take isolation precautions earlier, and start appropriate anti-tuberculous treatment to protect public health. It can be challenging to distinguish between diseases based on physical examination, radiological findings, and clinical features. For this reason,

the diagnostic algorithm can also include laboratory parameters understood to be supportive and distinctive.

Study Limitations

To fully evaluate our results, we must consider the limitations of this study. First, TB pneumonia patients were younger than CAP patients, few had advanced comorbidity, and the sample size was smaller.

Conclusion

Although additional studies with a higher number of TB pneumonia patients will provide us with more reliable information, it is important to correctly distinguish between TB pneumonia and CAP in countries where TB is common. Na, ALT, and BUN levels can be considered among the distinguishing laboratory parameters of the two diseases.

Ethics Committee Approval: The study is a single-center and retrospective study that was conducted after being approved by the University of Health Sciences Turkey, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital Scientific Board and Ethics Committee (approval number: 2021-102, date: 25.03.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Surgical and Medical Practices - S.T.O., S.N.S., M.G.O.; Concept - N.Ş.V., S.T.O., S.N.S., M.G.O.; Design - N.Ş.V., S.T.O., S.N.S., M.G.O.; Data Collection or Processing - N.Ş.V., S.T.O., H.A., K.K.; Analysis or Interpretation - F.T.A.; Literature Search - N.Ş.V.; Writing - N.Ş.V.

Conflict of Interest: No conflict of interest was declared by the authors.

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Comparison of Spinal Anesthesia vs. Local Infiltration Anesthesia in Postoperative Pain in Patients Undergoing Sinusectomy Surgery

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ABSTRACT

Introduction: Different anesthesia techniques were reported for the management of pilonidal sinus surgery, including local infiltration anesthesia (LIA), epidural anesthesia, spinal anesthesia (SA), and general anesthesia. However, even up to this date, the best anesthetic technique remains controversial. Here; we compared LIA SA for postoperative pain levels after pilonidal sinus disease surgery.

Methods: We conducted a single-center prospective observational clinical trial between May 2022 and January 2023 in our tertiary education hospital. A total of 60 patients with a diagnosis of pilonidal sinus were included in the study Postoperative 0-hour, 1 hour, 8 hours, and 24-hour numeric rating scores (NRS) of the patients were evaluated. Results were evaluated using Statistical Package for the Social Sciences.

Results: While 30 (50%) patients were operated under local anesthesia, 30 (50%) patients were operated under SA. 55 (91.7%) patients were male, 5 (8.3%) patients were female. The mean age of the patients operated under local anesthesia was 29.68±6.85 years, while the mean age of the patients operated under SA was 26.23±7.04 years. There was no statistically significant difference in age between the groups. When the comparison between postoperative NRS scores of SA and LIA was evaluated, there was no significant difference at postoperative 0 h and postoperative 1st hour. However, LIA NRS scores at 8th and 24th h were lower than SA scores. Recurrence was observed in 3 patients from both groups in the 3rd month outpatient controls. The difference between the groups was not statistically significant. All patients were discharged postoperatively on the 1st day of the surgery.

Conclusion: As a result, we may suggest using LIA for pilonidal surgery for the anesthetic technique.

Keywords: Spinal anesthesia, local infiltration anesthesia, sinusectomy surgery

Introduction

For 90% of anorectal interventions, the ambulatory approach was preferred among general surgery operations in our country (1,2). Different anesthesia techniques have been reported for the management of pilonidal sinus surgery, including local infiltration anesthesia (LIA), epidural anesthesia, spinal anesthesia (SA), and general anesthesia (1-5). But even up to date, the best anesthetic technique remains controversial.

Pilonidal sinus disease (PSD) was first reported in the 1880s, and different treatment possibilities have been described for PSD (6). PSD etiology was defined as chronic acquired inflammatory sickness that arises from the hair follicles of buttock cleft at the bottom of the spine with the incidence rate of 26/100,000 of the total population and the incidence was 2.5 times higher for males (7,8).

The study compared the postoperative pain level of patients with PSD under SA vs. LIA. The secondary aim of our study was to investigate any possible complications associated with anesthetic techniques or surgery.

Methods

We conducted a single-center prospective observational clinical trial between May 2022 and January 2023 in our tertiary education hospital. All patients' consent was taken from them. The study was approved by the University of Health Sciences Turkey, İstanbul Training and

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Research Hospital Local Ethics Committee (approval number: 143, date: 06/05/2022). We included patients aged between 18-65 years, American Society of Anesthesiologists I-III (ASA I-III), patients planning to undergo pilonidal sinus surgery, and patients who accepted to join our study. We excluded patients with a change of surgical technique in the operation or a change of anesthetic technique, allergy to local anesthetics, patients with bleeding disorders, patents with infection at the site of lumbar region from whom SA was planned, septic patients, and serious aortic valve stenosis.

The Anesthesia Technique

Two groups of anesthesia techniques were planned for the study. The first group (group S) SA was administered for the surgery. A second group (group L) LIA was planned for the surgery. All patients had standard ASA suggested monitorization with non-invasive blood pressure, heart rate, and pulse oximetry, and they were given iv. 2 mg of midazolam before surgery sedation. We administered a local anesthetic solution of 10 mL 2% lidocaine +20 mL bupivacaine 0.5% at the prone position. For the anesthetic technique of group S, SA was made at L3-L4 or L4-L5 levels with bupivacaine heavy 3.0 mL 0.5%. A 25- or 26-G spinal needle was used to enter the subrachnoid space. The patient was turned to prone position.

Pain assessment was performed numeric rating score (NRS) pain scores were examined at 0, 1, 8 and 24 h after surgery. Evaluation of 0-10 (0: No pain, 10: Most severe pain) by asking the patient at the hour and evaluating it with an anesthesia doctor who was blinded to the study groups.

We administered 1 g paracetamol during the operation period to both patient groups for analgesia. Non-steroid analgesics were prescribed for pain management during the postoperative period, and the patient was discharged.

Surgical Technique

Patients with infected pilonidal sinus, patients with more than 4 orifices, and patients with a distance between orifices of more than 8 cm were excluded from the study. The limited excision (sinusectomy) surgical technique described by Soll et al. (9) was applied to all patients. The patients were placed in a prone position on the operating table. The intergluteal fold was separated by tape. Sinus staining was achieved by injecting diluted methylene blue through the pilonidal sinus orifices. The orifices and sinuses were excised. After hemostasis the wound was left open for secondary healing. Patients were instructed to wash their wounds at least twice a day. The patients were checked in the outpatient clinic (9).

Statistical Analysis

Data analysis was done with the Statistical Package for the Social Sciences (SPSS version 26.0, IBM, Armonk, NY, USA) program. The distribution of variables was measured using the Kolmogorov-Smirnov test. If continuous data were normally distributed, they were expressed as mean ± standard deviation (SD), otherwise as a median-interquartile range. Descriptive statistics, numbers, and percentages for categorical variables and mean, SD, median, minimum, and maximum for numerical variables were given. Comparisons of numerical variables in two independent groups were made with Student's t-test for the variable satisfying the normal distribution condition and with the Mann-Whitney U test when the normal distribution. P-value <0.05 was accepted as statistically significant. Chi-square test was used in qualitative data.

Results

A total of 60 patients with a diagnosis of pilonidal sinus were included in the study. While 30 (50%) patients were operated under local anesthesia, 30 (50%) patients were operated under SA. All patient demographic data are presented in Table 1. Postoperative 0-, 1-, 8-, and 24-hour NRS scores of the patients were evaluated. While 55 (91.7%) patients were male, 5 (8.3%) were female. Of the patients operated under local anesthesia, 28 (93.3%) were male and 2 (6.7%) were female. Of the patients who were operated under SA, 27 (90%) were male and 3 (10%) were female. The mean age of the patients operated under local anesthesia was 29.68±6.85 years, while the mean age of the patients operated under SA was 26.23±7.04 years (Table 2). There was no significant difference age between groups (p=0.716).

When the comparison between postoperative NRS scores of SA and LIA was evaluated, there was no significant difference at postoperative 0 hour (p=0.830) and postoperative 1st hour (p=0.172). However, we have reported a statistically significant difference in terms of NRS scores at the 8th hour (p=0.002) and 24th hour (p=0.015). LIA NRS scores were significantly lower than SA scores (Table 2).

Recurrence was observed in 3 patients from both groups in the 3rd month outpatient controls. It was statistically not significant. All patients were discharged postoperatively on the first day of surgery. We did not encounter any anesthesia-related complications.

Discussion

In this study, we reported a significant difference in pain scores for LIA than SA at 8th and 24th hour. Both techniques had comparable pain scores during the early postoperative period because the effect of SA persisted at that period. However, starting from the 8th hour, the applied LIA technique demonstrated improved pain levels. With this technique, we did not encounter any possible SA-associated complications and we think that using the LIA technique with better pain scores and less complications should be suggested.

Table 1. Demographic data

Age (mean ± SD) (years)	**25.91±6.85**
Sex (%)	
Male	55 (91.7%)
Female	5 (8.3%)
Anesthesia type	
Spinal anesthesia	30 (50%)
Local infiltration anesthesia	30 (50%)
ASA score	
I	-
II	-

SD: Standard deviation, ASA: American Society of Anesthesiologists

Table 2. Comparison of variables

	Local Infiltration anesthesia (n=30)	Spinal anesthesia (n=30)	p-value
Age (mean ± SD) (years)	26.6±6.7	26.23±7.04	0.716
Sex (%)			
Male	28 (93.3%)	27 (90%)	1.00
Female	2 (6.7%)	3 (10%)	
NRS score 0. hour (mean ± SD)	1.53±1.27	2.10±2.45	0.830
NRS score 1. hour (mean ± SD)	1.43±1.33	2.30±2.18	0.172
NRS score 8. hour (mean ± SD)	1.57±1.40	3.47±2.50	**0.002***
NRS score 24. hour (mean ± SD)	1.33±1.52	2.80±2.54	**0.015***
Recurrence	3 (10%)	3 (10%)	1.00

*P-value <0.05 was accepted as statistically significant. SD: Standard deviation, NRS: Numeric rating score

A recent study by Barada et al. (4) investigated the effectiveness of sacrococcygeal local anesthesia for complicated pilonidal surgery in 394 patients. They reported lower post-operative pain scores and analgesic consumption for using LIA (4). Similar to our findings, they also. suggested to use LIA to eliminate the risk of complications associated with general and SA (4,10). There are studies in the literature operated their pilonidal sinus surgeries with LIA and they all report favorable results (11,12).

Sungurtekin et al. (1) compared the local anesthesia technique with sedation combination and SA for ambulatory pilonidal disease. They concluded that LIA with sedation resulted in a faster hospital stay and diminished hospital expenses, without any serious side effects when compared with SA (2). We reported similar results with their findings, but our LIA patients pain scores were even better.

Although we did not see any SA-related complications in our study, the use of SA for ambulatory surgery may end up with the possibility of facing postural spinal headache, temporary radicular irritation, and even urinary retention (1,13). In addition to that transient neurologic symptoms may occur after the administration of SA (1,14).

In terms of surgical complications, we found similar results between the groups. Both groups had a %10 recurrence rate, and we did not encounter any other surgical complications. In a recent study, Garg and Yagnik (6) reported a 3.7% recurrence rate, which was less than our results. However, in the literature, there are studies reporting a recurrence rate for pilonidal sinus surgery between 0-11.9% for excision with open healing treatment and between 0-20% for excision with midline closure treatment or between 0-11% for midline closure with different flaps (6,15-17). These results were similar to our study outcomes. In a study investigating the recurrence rate of the pilonidal sinus between general and SA groups, the authors could not show any association with anesthesia type (18).

Study Limitations

We have some limitations for our study; first, our sample size was small, and a larger sample size could give more precise results. Second, we used NRS for evaluating pain level of the patients and it is a subjective test battery for the assessment. But to date it has been widely used in many studies. The third one is that this research was a single-centered study.

Conclusion

We have reported improved results for LIA when we compared it with SA. Pilonidal surgery our patients whom we administered LIA had lower pain scores at 8th and 24th hours and two groups showed similar complications. As a result, we may suggest using LIA for pilonidal surgery for the anesthetic technique.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 143, date: 06/05/2022).

Informed Consent: All patients' consent was taken from them.

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Investigation of the Risk of Diabetic Foot Ulcers and Affecting Factors in Patients with Type 2 Diabetes

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ABSTRACT

Introduction: patients with diabetes are at risk of developing diabetic foot ulcers (DFUs), which can lead to serious consequences such as the loss of a limb. This study investigated the DFU risk of patients with type 2 diabetes (T2D) and to examine the factors affecting it.

Methods: This cross-sectional study was conducted using patients aged 18 and over who had T2D for at least one year and without DFU. The Patient Information Form and the Turkish Version of the Brief Diabetic Foot Ulceration Risk Checklist (BDURC-TR) were used to obtain data. Anthropometric measurements, levels of glycosylated hemoglobin A1c, and fasting plasma glucose were recorded.

Results: The total BDURC-TR score of the 150 participants was 1.81±1.42 and 11.3% (n=17) had a score of ≥4. The BDURC-TR score was statistically significantly ≥4 in those with known diabetes-related complications, those using combined diabetes treatment, those with long diabetes duration, and those with greater height ($p<0.001$; $p=0.033$; $p=0.004$; $p=0.013$, respectively). Although not significant according to the cut-off values, there was a statistically significant correlation between the BDURC-TR total score and age, weight, and waist circumference values ($r=0.246$, $p=0.002$; $r=0.0163$, $p=0.046$; $r=0.182$, $p=0.026$, respectively). The BDURC-TR total score was also higher in men and in those using additional drugs ($p=0.037$ and $p=0.024$).

Conclusion: Our study showed that 11.3% of the patients with T2D had a high DFU risk. The presence of diabetes-related complications, combined diabetes treatment, a long duration of diabetes, and having greater height was high-risk factors for DFU.

Keywords: Complications, diabetic foot ulcers, diabetic foot ulcer risk checklist, type 2 diabetes

Introduction

Diabetic foot ulcer (DFU) is characterized by ulceration, infection, and/or gangrene associated with diabetic neuropathy (DNP) and peripheral vascular disease in the foot (1). The risk of DFU occurrence in patients with diabetes is approximately 15-25% (2).

The DFU is the most common complication of diabetes that is difficult to treat and causes hospitalizations (3). It results in disability, loss of workforce, decreased the quality of life, and increased health care costs. Prolonged life expectancy and years with diabetes increase the risk of developing DFU (4).

The lifetime risk of DFU is also rising with increased medical complexity in people with diabetes. Therefore, the development of DFU can be prevented by keeping diabetes under control and providing foot care education to patients. High-risk patients should be identified and followed more frequently with both routine examination and risk assessment for DFU (5).

This study investigated the DFU risk of patients with type 2 diabetes (T2D) and examine the factors affecting it.

Methods

Study Design and Ethical Approval

This study was designed as a single-centered and cross-sectional study. Ethical permission to conduct this study was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 379, date: 24.11.2021). The study was conducted under the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

The selection and Description of Participants

Study Population

All participants were selected from patients with T2D and were referred to the family medicine outpatient clinic of a tertiary hospital from from

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December 2021 to April 2022 for any reason. The study included 150 patients aged 18 and over who had a known diagnosis of T2D for at least one year, did not have been diagnosed with DFU, could understand, answered the questions asked, was literate, and agreed to participate in the study.

The sample size was calculated with the simple random sampling method from the study population, and when the incidence of DFU was considered 0.15 at the α effect level of 0.05, the minimum number of participants required for the study was 110 with a 95% confidence interval.

Exclusion Criteria

Patients under the age of 18, those with a disability to communicate (hearing and speech disorders, cognitive dysfunction, uncooperative), gestational diabetes, type 1 diabetes (T1D), T2D diagnosis less than 1 year ago, and illiterates were excluded.

Data Collection Tools

Patient Information Form

A patient information form was formulated that questioned the participants' sociodemographic characteristics (age, gender), diabetes characteristics (duration, treatment type, presence of known (physician-diagnosed) diabetes-related complications, hospitalization in the last year, treatment compliance), presence of comorbidities, and smoking history. Compliance with treatment was determined according to the patient's statement. Arterial blood pressure (mmHg), waist circumference (WC) (cm), height (m), weight (kg), and body mass index (BMI, kg/m^2) were measured and recorded. The levels of glycosylated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) obtained from venous blood were recorded.

The Turkish Version of Brief Diabetic Foot Ulceration Risk Checklist

The Brief Diabetic Foot Ulceration Risk Checklist (BDURC) was developed by Zhou et al. (6) in 2018 to determine the risk of DFU in diabetic patients. The Turkish validity and reliability study of the scale was performed by Dincer et al. (7) in 2021. BDURC-TR was composed of 12 items and 2 factors. The questions in the scale can be answered as "Yes" or "No." The "Yes" answer is scored as 1 point and the "No" answer as 0 points. A total of 0-12 points can be obtained from the scale. An increase in scores indicates an increased risk of DFU. The Cronbach's alpha coefficient of the Turkish version was determined to be 0.79 (7). The cut-off point for DFU risk was determined as 4 in the original study (6).

Statistical Analysis

The IBM SPSS Statistics 25.0 program was used for statistical analysis. Descriptive data on the sociodemographic information of the participants were presented as number (%) and mean ± standard deviation tables. When the study data were analyzed in terms of normality assumptions, Kolmogorov-Smirnov values were determined as $p<0.05$. Spearman correlation analysis, one of the non-parametric tests, was performed to investigate the relationship between BDURC scores and various numerical variables. The Mann-Whitney U test was applied to determine whether there was a significant difference between BDURC scores and various numerical variables of the participants. Chi-square test was used for comparison of categorical variables. $p<0.05$ was considered statistically significant.

Results

The ages of 150 participants in the study ranged from 33 to 88 years, with a mean value of 60.37±10.56. The total score from BDURC-TR was 1.81±1.42 (0.00-6.00). 11.3% of the participants (n=17) had a BDURC-TR total score of ≥4. The distribution of baseline characteristics of the participants is presented in Table 1.

The BDURC-TR score was statistically significantly ≥4 in patients with a long diabetes duration and those with greater height ($p=0.004$ and $p=0.013$). The data of the participants on laboratory examinations and anthropometric measurements and the distribution of data according to BDURC-TR risk groups are summarized in Table 2.

As seen in Table 3, in patients with known diabetes-related complications and in those using combined therapy, the BDURC-TR score was statistically significantly ≥4 ($p<0.001$ and $p=0.033$). All participants who had a BDURC-TR total score of ≥4, were determined to have a chronic disease other than diabetes. But it was not found statistically significant ($p>0.05$). The distribution of the participants' medical characteristics according to the BDURC total score and risk groups are analyzed in Table 3.

Although not significant according to the cut-off values, there was a statistically significant difference between the BDURC-TR total score and age, weight, and WC of the participants ($r=0.246$, $p=0.002$; $r=0.0163$, $p=0.046$; $r=0.182$, $p=0.026$, respectively). The BDURC-TR total score was higher in men and in those using additional drugs ($p=0.037$ and $p=0.024$).

Discussion

Main Findings

In this study, which aimed to examine the DFU risk and affecting factors in patients with T2D, 11.3% of the patients with T2D were found to have a high risk of DFU. The presence of diabetes-related complications, combined therapy, long duration of the diabetes, and greater height were determined to be DFU risk factors regarding BDURC-TR cut-off levels. Gender, age, weight, and WC were also influential factors on the BDURC-TR total score.

Comparison with Existing Literature

The reported incidence and prevalence of DFU vary widely depending on the study design, population, and era. They are also affected by differences in DFU definitions. As is known, DFU may develop in 15-25% of patients with diabetes (2). Although the global prevalence of DFU varies between countries, it has been reported to be between 1.5 and 16.6% (8). In the BDURC development study, the one-year incidence of DFU was found to be 3.6% in T2D patients followed for 1 year, and the scale total score was 4.2±2.3 (6). In a multicenter study investigating the incidence of DFU in patients with T2D and a new foot ulcer, the annual incidence of a new DFU was 0.42% (9). In the study in which BDURC was adapted into Turkish, it was reported that 86.7% of the patients had a total scale score of 4 and above, which is different from the literature (7).

In our study, 17 patients (11.33%) were determined to be at high risk (scored 4 or more on the BDURC-TR) for DFU. Studies measuring DFU risk using BDURC are limited in the literature. Since it is a scale that evaluates the risk of diabetic foot in Turkish people and has a multidisciplinary team approach, BDURC-TR was used in this study. Although there is a small number of people with DFU risk according to the cut-off level of the BDURC-TR, it can be said that it is compatible with the literature when compared to the number of all participants. This result also shows the importance of risk screening for DFU, which can lead to important results such as loss of a limb. It should be noted that DFU-specific instruments to be used in risk assessment may include more clinical aspects of DFU and will be more sensitive to disease-related changes than generic tools.

In the literature, many factors have been shown to be associated with the risk of DFU. Demographic, socioeconomic, and metabolic factors are also strongly related to DFU. There are many studies indicating that the

Table 1. Baseline characteristics of the study participants (n=150)

		Min.-Max.	Mean ± SD
Age (years)		33-88	60.37±10.56
Diabetes duration (years)		1.00-35.00	10.19±6.78
BDURC-TR total score		0.00-6.00	1.81±1.42
Total score<4 (n=133)		0.00-3.00	1.46±1.06
Total score ≥4 (n=17)		4.00-6.00	4.52±0.71
		n	**%**
Gender			
	Female	94	62.7
	Male	56	37.3
Smoking status			
	Active smoker	27	18.0
	Ex-smoker	33	22.0
	Non-smoker	90	60.0
Presence of comorbidities			
	No	13	8.7
	Yes	137	91.3
Additional drug use			
	Yes	134	89.3
	No	16	10.7
Presence of complication due to diabetes			
	No	93	62.0
	Yes	57	38.0
Complication due to diabetes (n=57)*			
	Retinopathy	23	27.4
	Nephropathy	56	66.7
	Neuropathy	5	6.0
History of hospitalization due to diabetes in last year			
	No	144	96.0
	Yes	6	4.0
Diabetes treatment type*			
	OAD	61	40.7
	Insulin	17	11.3
	Combined therapy	72	48.0
Compliance with treatment			
	Yes	106	70.7
	Partially	28	18.7
	No	16	10.7

Data presented as min.-max., mean (SD), n and %. BDURC-TR: The Turkish version of Brief Diabetic Foot Ulceration Risk Checklist, OAD: Oral anti-diabetic drug, SD: Standard deviation.
*As the questions can contain multiple answers, the number of (n) exceeds the sample size

risk of DFU increases with age and years of living with diabetes (10,11). In addition, a statistically significant relationship has been revealed between being 60 years of age and older and DFU (12).

Similar to the literature, the present study determined that the risk of DFU increased with age and disease duration. The incidence of complications increases with the prolongation of life expectancy and therefore the time spent with diabetes. Adequate metabolic control is required to reduce the cumulative effects of hyperglycemia and micro- and macrovascular complications as age and duration of diabetes increase.

Major comorbidities increase the risk of DFU and other diabetes-related complications (13,14). It has been shown that coronary artery disease has a significantly higher prevalence in patients with DFU because of a combination of cardiovascular risk factors (15). Studies have also revealed a positive relationship between hypertension and DFU (16). In addition, some studies have reported that dyslipidemia is a risk factor for DFU development (17).

In our study, the majority of the participants had any chronic disease and all the participants who had a BDURC-TR total score of ≥4, were determined to have a chronic disease other than diabetes. However, it was not statistically significant. It is thought that the cut-off value of the scale we used effects obtaining different results from the literature in this context.

Diabetes-related complications are the most important basic risk factors for DFU. Since their symptoms are not obvious in the first stage, the risk of DFU may increase further as patients may be overlooked (18). The combination of neuropathy and peripheral arterial disease has been associated with an increased risk of ulcers in most previous studies (5). In a multicenter study, patients with diabetes-related complications were determined to have a higher risk of DFU (19).

Similar to the literature, the present study demonstrated that having DM complications other than DFU increased the risk of DFU. Since DFU

Table 2. Data on participants' laboratory findings and anthropometric measurements according to risk groups

	Findings according to BDURC-TR total scores		
	Total score <4, (n=133)	Total score ≥4, (n=17)	
	Mean ± SD	Mean ± SD	p
Age (years)	59.86±10.41	64.35±11.18	0.120
DM duration (years)	9.62±6.47	14.64±7.68	**0.004**
FPG (mg/dL)	202.14±91.98	204.24±91.56	0.760
HbA1c (%)	8.92±2.23	9.35±2.92	0.727
Height (m)	1.61±0.08	1.68±0.09	0.013
Weight (kg)	80.68±15.24	86.00±14.42	0.142
WC (cm)	107.19±12.07	111.59±12.74	0.251
BMI (kg/m^2)	30.92±5.95	30.49±4.88	0.769
HR (rate/min)	75.41±7.53	79.53±8.74	0.071
SBP (mmHg)	126.58±17.70	124.12±15.12	0.523
DBP (mmHg)	77.17±8.48	75.59±7.04	0.476

Mann-Whitney U test, Data presented as min.-max. and mean (SD). BDURC-TR: The Turkish Version of Brief Diabetic Foot Ulceration Risk Checklist, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, HBA1c: Hemoglobin A1c, HR: Heart rate, SBP: Systolic blood pressure, SD: Standard deviation, WC: Waist circumference

Table 3. Comparison of BDURC risk groups according to clinical variables of the participants

		BDURC-TR		
		Total score <4, (n=133)	Total score ≥4, (n=17)	p
		n (%)	n (%)	
Gender	Female	86 (64.7%)	8 (47.1%)	0.158[a]
	Male	47 (35.3%)	9 (52.9%)	
Smoking status	Active smoker	24 (18.0%)	3 (17.6%)	1.000[b]
	Ex-smoker	29 (21.8%)	4 (23.5%)	
	Non-smoker	80 (60.2%)	10 (58.8%)	
Groups according to BMI	Normal	17 (12.8%)	3 (17.6%)	0.787[a]
	Overweight	43 (32.3%)	6 (35.3%)	
	Obese	73 (54.9%)	8 (47.1%)	
Presence of comorbidities	No	13 (9.8%)	0 (0.0%)	0.363[b]
	Yes	120 (90.2%)	17 (100.0%)	
Presence of diabetes-related complications	No	90 (67.7%)	3 (17.6%)	**<0.001[a]**
	Yes	43 (32.3%)	14 (82.4%)	
Diabetes treatment*	OAD	57 (42.9%)	4 (23.5%)	**0.033[a]**
	Insulin	17 (12.8%)	0 (0.0%)	
	Combined therapy	59 (44.4%)	13 (76.5%)	
Compliance with treatment	Yes	96 (72.2%)	10 (58.8%)	0.421[b]
	Partially	24 (18.0%)	4 (23.5%)	
	No	13 (9.8%)	3 (17.6%)	

Data presented as n (%). [a]Pearson chi-square, [b]Fisher's exact test, p<0.05. BMI: Body mass index, BDURC-TR: The Turkish Version of Brief Diabetic Foot Ulceration Risk Checklist, OAD: Oral antidiabetic drug, SD: Standard deviation. *As the questions can contain multiple answers, the number of "n" exceeds the sample size

and other DM-related complications act synergistically in contributing to clinical outcomes and morbidity, their development should be prevented with multidisciplinary DM care and risk assessments.

Generally, the male gender is a crucial risk factor for DFU (13,15). However, there are also studies indicating no significant relationship between gender and DFU (20,21).

As in many previous studies, the risk of DFU was also higher in men (regarding BDURC total score) in our study. Sex differences could be explained by underlying risk factors, attitudes about footwear and footcare, and adherence to the treatment.

Glycemic control is one of the most critical factors in DFU development and glycemic disorders increase the risk of DFU. Dekker et al. (16) revealed that diabetic patients with foot ulcers had higher averages of HbA1c than those without foot ulcers, as well as a higher cumulative glycemic load. Similarly, mean HbA1c values were found to be statistically significantly higher in diabetic patients with DFU. On the other hand, the DFU risk of those with HbA1c values above 9% was significantly higher than those with HbA1c values lower than 6.5%. There was no significant increase in DFU risk in those with HbA1c values between 6.5 and 9% compared to those with HbA1c values lower than 6.5% (22). Also, studies showed that patients with high plasma glucose levels have a higher risk of developing DFU in the future (23,24).

Contrary to previous studies, in our study, there was no statistically significant difference between FPG and HbA1c from DFU risk. This suggests that glycemic control alone may not be a responsible factor in the development of DFU and may be due to differences in other variables (patient-and foot-specific factors) of the participants.

In many studies, a significant relationship was reported between insulin use and DFU. Yazdanpanah et al. (25) determined in their two-year follow-up study that patients treated with insulin were more likely to develop DFU than patients treated with oral anti-diabetic drug (OAD) or lifestyle changes alone. In a cohort study, insulin and combined therapy (insulin and OAD) were found to be associated with DFU risk, but there was no significant relationship between OAD and DFU (26).

In our study, those using combined therapy had a higher risk of DFU. Considering that patients with poor glycemic control cannot be achieved with OAD, they are switched to combined therapy. It should be kept in mind that these patients have poor glycemic control, which increases the risk of DFU.

Although obesity is one of the main risk factors for developing T2D, its contribution to DFU development risk is still controversial. There are studies in the literature revealing that obesity increases DFU risk (15). In fact, obesity increases the likelihood of developing DFU by 2.1 times (27). In contrast, according to recent systematic reviews, obesity is not associated with incident or recurrence of DFU, amputation, or mortality (28). Furthermore, increased WC was indicated to be a risk factor for DFU (29).

Similar to the literature, there was no significant relationship between BMI and DFU risk in our study. However, a significant correlation was found between weight and WC, which are critical components of obesity, and the risk of DFU.

It is thought that the length of the nerve roots is an important factor in the development of neuropathy due to DNP, that the long nerve roots are affected early by degeneration, and therefore the risk of neuropathy increases as the height of the patients increases (30).

In support of this, our study observed a significant correlation between height and DFU risk. In fact, the correlation of the total score with weight, height, and WC could also be due to different gender compositions in subjects with high versus low DFU risk.

Study Limitations

This study has some limitations. First, due to the single-center and cross-sectional design and relatively small sample size of the study, the findings may not be generalized to the population. Second, we only included T2D patients because we predicted that we would not be able to reach sufficient T1D patients. Lastly, the participants were not followed-up. Contributions to the literature should continue with a larger sample, more comprehensive, and multicenter studies, including patients with T1D and newly diagnosed T2D.

Conclusion

Our study showed that 11.3% of the patients with T2D had a high DFU risk. The presence of diabetes-related complications, combined diabetes treatment, long duration of diabetes, and having greater height were high-risk factors for DFU. Gender, age, weight, and WC were also influential factors on the DFU risk. These factors should be considered to prevent the formation of DFU. In addition to routine evaluations, patients with T2D should be examined periodically in terms of DFU risk with DFU-specific risk assessment methods, and high-risk patients should be followed more frequently. Modifiable risk factors should be eliminated by providing metabolic control. In-need referral to a higher level of healthcare can save both the leg and the life of the patient.

Ethics Committee Approval: Ethical permission to conduct this study was obtained from the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 379, date: 24.11.2021). The study was conducted under the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all participants.

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Utilization and Effectiveness of the CA 19-9 Test for Cancer Diagnosis: Insights from Health Ministry Records

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ABSTRACT

Introduction: This retrospective study evaluated the use and findings of the CA 19-9 test in the diagnosis of pancreatic cancer.

Methods: The study analyzed data from a large population of 2,981,142 individuals who underwent CA 19-9 testing between 2017 and 2021. The number of tests performed, test rates per 100,000 population, and 25,808,137 test results were assessed. The study also investigated the association between CA 19-9 levels and cancer diagnoses.

Results: The study found that CA 19-9 was a widely used tumor marker for pancreatic cancer diagnosis. However, it was noted that the test had limitations as a standalone diagnostic tool. In the analyzed population, the test results showed elevated CA 19-9 levels in more than 80% of patients diagnosed with pancreatic cancer. The study also observed variations in test use and outcomes across different age groups, genders and regions.

Conclusion: Our findings highlight the significance of the CA 19-9 test in the diagnosis of pancreatic cancer. However, it is crucial to consider the limitations of using CA 19-9 as a standalone test and incorporate it into a comprehensive diagnostic approach involving other clinical and laboratory methods. Further multicenter and prospective studies are warranted to better understand the accuracy and effectiveness of the CA 19-9 test in pancreatic cancer diagnosis.

Keywords: CA 19-9, pancreatic cancer, tumor marker, diagnosis

Introduction

CA 19-9 is a tumor marker used in the diagnosis and treatment of cancer. Tumor markers are substances produced or secreted by cancer cells in the body and they assist in the diagnosis, staging and monitoring of treatment response in cancer. CA 19-9 is particularly a widely used marker in pancreatic cancer and bile duct cancer.

CA 19-9, also known as carbohydrate antigen 19-9, is a type of glycoprotein found on the cell surface. It is normally present at low levels in certain tissues (such as the pancreas, bile ducts, and intestines). However, cancer cells can produce this marker in excessive amounts, leading to its release into the bloodstream and detection at high levels (1).

The CA 19-9 tumor marker is used primarily in the diagnosis of certain cancer types, especially pancreatic cancer. Pancreatic cancer often does not present symptoms in its early stages, making it challenging to diagnose. CA 19-9 levels can provide a clue to the presence or progression of tumors like pancreatic cancer. Additionally, it can be used to monitor the response to treatment and assess the likelihood of disease recurrence. However, it is important to note that CA 19-9 alone is not sufficient as a diagnostic tool or cancer screening test. Elevated CA 19-9 levels can also occur due to other reasons. Therefore, CA 19-9 results should be interpreted in conjunction with other medical imaging tests and clinical evaluations. CA 19-9 is a cell surface glycoprotein produced by epithelial ductal cells in the pancreas, bile system, stomach, colon, uterus, and salivary glands. Its expression requires the presence of Lewis blood group antigens. Therefore, it is not a reliable marker for individuals with Lewis-negative phenotype (approximately 5-10% of the population) (2).

In addition to being excessively expressed in many benign and malignant gastrointestinal and extragastrointestinal tumors, CA 19-9 can also be positive in pancreatitis, pancreatic cysts, diabetes mellitus, liver cirrhosis, benign cholestatic diseases, and urological, pulmonary, and gynecological diseases (3).

CA 19-9 is a widely used tumor marker in the diagnosis of pancreatic ductal adenocarcinoma. Pancreatic cancer has a less than 7% 5-year survival rate and is the fourth most common cause of cancer-related deaths (4).

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CA 19-9 has been found to be elevated in more than 80% of patients diagnosed with pancreatic cancer. However, most international guidelines recommend using CA 19-9 in conjunction with the gold standard diagnostic method, pancreatic protocol CT, rather than relying on it alone (5). In benign pancreatic diseases such as pancreatitis and premalignant lesions such as intraductal papillary mucinous neoplasm, CA 19-9 can also be elevated in levels ranging from 10% to 50%. Therefore, it is not recommended to use CA 19-9 as a standalone test for diagnosing pancreatic cancer (6).

Despite these findings, once the diagnosis of pancreatic cancer is confirmed, CA 19-9 levels are crucial in determining appropriate staging and treatment regimens. Preoperative CA 19-9 levels are associated with prognosis.

CA 19-9 is a tumor marker that assists in the diagnosis and treatment of cancer. It is used in the diagnosis of certain cancer types, such as pancreatic cancer, and in monitoring the treatment response. However, CA 19-9 results should be interpreted alongside other medical findings and should not be used solely to establish a definitive diagnosis. The aim of this study was to investigate the diagnostic and prognostic value of CA 19-9 tumor marker levels in pancreatic cancer patients, while also assessing its potential utility in differentiating pancreatic malignancies from benign pancreatic diseases.

Methods

Data from a five-year period (2017-2021) were analyzed, including a total of 25,808,137 tests from 2,981,142 individuals. The test counts, test rates per population, and rates of exceeding the reference range were assessed based on gender, age groups, geographic regions, and healthcare institution types.

The CA 19-9 levels were determined using the immunoassay method and the results were transferred to the National Health Database, which is referred to as e-nabiz by the Ministry of Health. This database encompasses the health records of patients who have sought medical services from all healthcare institutions in Turkey, including their demographic characteristics, laboratory data, medication usage, comorbidities, and other health-related records.

The healthcare database service in Turkey is referred to as e-nabiz. The transmission of health data set packages is facilitated through XML web services. This database encompasses the health records of patients who have sought medical services from all public, private and university healthcare institutions in Turkey, including their demographic characteristics, laboratory data, medication usage, comorbidities, and other health-related records.

Ethical Considerations

The study adhered to ethical guidelines and protected the privacy and confidentiality of the individuals included in the data. University of Health Sciences Turkey, İstanbul Training and Research Hospital Institutional review board approval was obtained (approval number: 188, date: 21.07.2023), and all data were anonymized to ensure privacy.

Statistical Analysis

Descriptive statistics were used to analyze the data. The test counts, test rates per population, rates of exceeding the reference range, and cancer diagnosis rates were calculated and compared across different variables, including gender, age groups, geographic regions, and healthcare institution types.

Database and e-Pulse

e-Pulse is a platform developed by the Ministry of Health in Turkey that allows individuals to store and manage their health information digitally. For this study, patient information and health records were collected from the e-Pulse system. During the data collection process, personal information was protected and the principle of privacy was fully respected.

SKRS and ICD codes

SKRS is a data recording and reporting system used by the Ministry of Health in Turkey. This system aids in the more effective management of health services. In this study, data pulled from the SKRS, and ICD codes were used to analyze disease diagnoses, treatment plans, and the overall state of health services.

ICD codes are a standard disease and health problem classification system created by the World Health Organization and used worldwide. These codes are an important tool for identifying, monitoring, and treating diseases.

Data collection: Data were collected from medical records and laboratory databases. The information included demographics (gender, age), test requests, test results, cancer diagnoses, and healthcare institution types.

The study population: The study population consisted of individuals who underwent CA 19-9 testing during the study period. Both men and women were included in the analysis.

Results

Between 2017 and 2021, CA 19-9 tests were requested from 4,018,913 individuals, with a total of 25,808,137 tests performed. This corresponds to an average of 6.42 tests per person, or 31,230 tests per 100,000 population. Among the tumor markers used in our CA 19-9 study, it ranks second in terms of tests performed per 100,000 population.

When comparing the number of CA 19-9 tests over the years, there is an increasing trend in the number of tests and tests per 100,000 population from 2017 to 2019. However, there is a significant decrease in test numbers in 2020 and 2021 (Table 1).

In females, the number of test requests shows a similar pattern to the general population, with an increasing trend from 2017 to 2019 and a significant decrease in 2020 and 2021. Throughout the years, CA 19-9 ranks fourth among tumor markers tested in females. In males, there is also an increasing trend in test requests from 2017 to 2019, followed by a notable decrease in 2020 and 2021. When comparing the ratio of female-to-male test requests, the ratio was 1.94 in 2017, 1.93 in 2018, 1.91 in 2019, 1.77 in 2020 and 1.84 in 2021 (Table 2).

Table 1. Number of tests and the ratio of the population by years

	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
CA 19-9	4,944,869	6,119	5,629,873	6,865	6,168,998	7,419	4,374,951	5,232	4,689,446	5,608

Table 2. Number of test requests by years

Women										
Year	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
CA 19-9	3,267,947	8,114	3,710,145	9,079	4,053,869	9,784	2,800,616	6,716	3,039,855	7,290
Men										
Year	2017		2018		2019		2020		2021	
	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population	Number of tests	Number of tests per 100,000 population
CA 19-9	1,676,922	4,137	1,919,728	4,666	2,115,125	5,070	1,574,335	3,756	1,649,591	3,935

Table 3. Number of test requests by years in age groups

	2017			2018			2019			2020			2021		
CA 19-9	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+
	52,631	3,510,673	1,381,565	55,070	3,981,668	1,593,132	56,853	4,314,922	1,797,221	31,943	3,059,942	1,283,061	30,899	3,231,224	1,427,323
	2017			2018			2019			2020			2021		
	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+	0-17	18-64	65+
	230	6,879	20,036	240	7,672	22,169	249	8,183	23,802	140	5,783	16,132	136	6,107	17,946

Test consumption per 100,000 persons by years and age groups

Regarding age groups, CA 19-9 is most frequently requested in the 18-64 age range, followed by the 65 and older age groups, and it is least frequently requested in the 0-17 age group. The ratio of test requests between the 18-64 age group and the 65 and older age group was 2.54 in 2017, 2.49 in 2018, 2.40 in 2019, 2.38 in 2020, and 2.26 in 2021.

The rate of test consumption per 100,000 population between the 18-64 age group and the 65 and older age group was 1/2.91 in 2017, 1/2.88 in 2018, 1/2.90 in 2019, 1/2.78 in 2020 and 1/2.93 in 2021. Among the 65 and older age groups, CA 19-9 ranks as the third highest tumor marker in test consumption per 100,000 population From 2017 to 2019, test requests increased across all age groups, followed by a significant decrease in 2020 and 2021 (Table 3).

When comparing the rates of cancer diagnosis at any given time for individuals who underwent CA 19-9 testing, the rate of cancer detection increased as the years progressed, with 28% of individuals diagnosed with cancer in 2017, 38% in 2020, and 33% in 2021. Among individuals tested for tumor markers, CA 19-9 ranks fifth in terms of the percentage of cancer diagnoses in 2017-2019 and fourth in 2020-2021.

Analyzing the timing of test requests in relation to the diagnosis, it is observed that in all years, a higher proportion of tests were requested before the diagnosis, followed by tests requested simultaneously with the diagnosis, and the least number of tests requested after the diagnosis. The ratio of test requests before diagnosis/simultaneous with diagnosis/after diagnosis was 1.95/1.60/1 in 2017, 4.14/1.32/1 in 2018, 6/1.37/1 in 2019, 8.55/1.71/1 in 2020, and 13.2/2.44/1 in 2021.

In 2017, 8.43% of individuals who underwent CA 19-9 testing received a cancer diagnosis associated with CA 19-9, while 30.69% received a cancer diagnosis not associated with CA 19-9. Until 2020, these percentages increased, with 16.60% of patients receiving a CA 19-9 associated cancer diagnosis and 42.22% receiving a cancer diagnosis not associated with CA 19-9. In 2021, these percentages were 10.76 and 39.21%, respectively. When comparing individuals diagnosed with CA 19-9 associated cancer and those diagnosed with non-CA 19-9 associated cancer, the ratio was 1/3.64 in 2017, 1/3.66 in 2018, 1/3.67 in 2019, 1/3.63 in 2020, and 1/3.64 in 2021 (Table 4).

In terms of test requests by geographic regions, the Marmara region had the highest number of CA 19-9 tests throughout the years. The Central Anatolia region ranked second with a ratio of 1.69 compared to the Marmara region in 2021. The lowest test request rate was observed in the Southeast Anatolia region, with a ratio of 11.81 compared to the

Marmara region. Similar to the overall trend in Turkey, there was an increase in test requests from 2017 to 2019 across all regions, followed by a notable decrease in 2020 and 2021 (Table 5).

When examining the number of tests per 100,000 population by cities, Istanbul had the highest number of test requests in all years, followed by Ankara. İzmir and Bursa ranked third and fourth, respectively. When analyzing the number of tests per 100,000 population, Sinop had the highest rate in 2017, Erzurum in 2018, Kırşehir in 2019, Isparta in 2020, and Karabük in 2021. İstanbul, Ankara, and İzmir, the top three cities with the highest number of test requests, were not among the top seven cities with the highest rate of tests per 100,000 population.

Comparing the clinics based on the number of test requests, in 2020, the medical oncology clinic had the highest number of test requests, whereas in other years, it was the internal medicine clinic. The second-highest number of test requests in 2020 was from the internal medicine clinic, and in other years, it was from the obstetrics and gynecology clinic. In 2017, medical oncology ranked fourth, in 2018-2019 it ranked third, in 2020 it was first, and in 2021 it ranked second. Family medicine ranked seventh in 2017 and 2018, sixth in 2019, seventh in 2020, and sixth in 2021. The emergency medicine clinic consistently ranked eighth in all years (Table 6).

Table 4. Distribution of cancer diagnosis related to CA 19-9

Year	Related cancer diagnosis		Non-related cancer diagnosis		Total number of people tested
2017	85,579	8.43%	311,759	30.69%	1,015,684
2018	100,048	8.69%	367,152	31.89%	1,151,453
2019	109,558	8.88%	402,246	32.61%	1,233,576
2020	102,848	11.60%	374,276	42.22%	886,560
2021	103,821	10.76%	378,276	39.21%	964,696

Table 5. CA 19-9 geographical distribution by years and number of test requests

Region	Number of tests, 2017	Region	Number of tests, 2018	Region	Number of tests, 2019	Region	Number of tests, 2020	Region	Number of tests, 2021
Marmara region	1,814,418	Marmara region	2,024,210	Marmara region	2,232,971	Marmara region	1,602,414	Marmara region	1,701,631
Central Anatolia region	863,701	Central Anatolia region	1,053,762	Central Anatolia region	1,236,213	Central Anatolia region	898,203	Central Anatolia region	1,003,159
Aegean region	712,908	Aegean region	767,383	Aegean region	841,814	Aegean region	633,179	Aegean region	674,803
Black sea region	479,400	Black sea region	553,447	Mediterranean region	616,861	Mediterranean region	421,600	Mediterranean region	433,103
Eastern Anatolia region	446,903	Mediterranean region	540,429	Black sea region	556,862	Black sea region	387,036	Black sea region	422,987
Mediterranean region	417,829	Eastern Anatolia region	450,477	Eastern Anatolia region	448,581	Eastern Anatolia region	284,308	Eastern Anatolia region	309,750
Southeast Anatolia region	209,710	Southeast Anatolia region	240,162	Southeast Anatolia region	235,676	Southeast Anatolia region	148,140	Southeast Anatolia region	143,967

Table 6. CA 19-9 top 10 clinics by years and number of test requests

2017		2018		2019		2020		2021	
Internal medicine	1,169,349	Internal medicine	1,291,526	Internal medicine	1,362,180	Medical oncology	905,912	Internal medicine	988,546
Gynecology and obstetrics	1,034,700	Gynecology and obstetrics	1,165,524	Gynecology and obstetrics	1,232,161	Internal medicine	850,350	Medical oncology	860,051
General surgery	633,224	Medical oncology	775,934	Medical oncology	923,263	Gynecology and obstetrics	798,103	Gynecology and obstetrics	832,797
Medical oncology	638,285	General surgery	697,597	General surgery	701,112	General surgery	471,606	General surgery	493,896
Gastroenterology	326,143	Gastroenterology	374,140	Gastroenterology	374,381	Gastroenterology	288,781	Gastroenterology	326,140
Radiation oncology	148,820	Radiation oncology	174,606	Family medicine	203,648	Radiation oncology	111,698	Family medicine	130,095
Family medicine	131,158	Family medicine	169,700	Radiation oncology	181,935	Family medicine	111,675	Radiation oncology	110,971
Emergency medicine	85,613	Emergency medicine	94,579	Emergency medicine	130,913	Emergency medicine	104,800	Emergency medicine	98,428
Chest medicine	63,870	Neurology	72,895	Gynecological oncology surgery	92,246	Gynecological oncology surgery	70,571	Gynecological oncology surgery	87,855
Urology	61,921	Urology	69,979	Neurology	87,209	Neurology	61,290	Neurology	71,451

When examining the diagnoses entered in the test application, "abdominal pain unspecified" was the most frequently entered diagnosis in 2017-2018, followed by "unspecified diagnosis," and in 2019-2021, the most frequently entered diagnosis was "vitamin D deficiency, unspecified." In the five-year period, "vitamin D deficiency, unspecified" was the most frequently entered diagnosis, followed by "abdominal pain, unspecified" and "essential (primary) hypertension" ranked third.

Regarding the rates of exceeding the reference range of the test, the highest rate was in 2020 at 13.23% and the lowest rate was in 2018 at 10.39%. When comparing the rates of exceeding the reference range by healthcare facility level, the highest rate of 12.75% was observed in tertiary level hospitals, followed by 9.95% in secondary level hospitals, and the lowest rate of 4.87% in primary care facilities. Analyzing the rates of exceeding the reference range by healthcare facility type, the overall rate was 11.56%, with the highest rate of 14.70% in university hospitals, followed by 13.94% in private healthcare institutions, and 9.73% in public hospitals.

When examining the rates of exceeding the reference range by geographical regions, the highest rate of 13.5% was observed in the Mediterranean region, which ranked second in the number of test requests and tests per 100,000 population throughout 2019-2021. The Ege region ranked second with a rate of 12.4%, and the lowest rate of 10.1% was observed in the Eastern Anatolia region. When comparing the rates of exceeding the reference range by gender, the overall rate was 11.53%, with a higher rate of 14.87% in males and a lower rate of 9.62% in females. Analyzing the rates of exceeding the reference range by age groups, the highest rate of 16.66% was observed in the 65 and older age group, followed by 9.31% in the 18-64 age group, and 8.86% in the 0-17 age group. When comparing age groups, the ratio of positive results was calculated as 1.88/1.05/1.Regarding the rates of exceeding the reference range by admission status, the highest rate of 21.31% was observed in inpatient cases, followed by 12.01% in outpatient cases, and 10.59% in day cases. When comparing the rates, the ratio was calculated as 2.01/1.13/1. When examining the rates of exceeding the reference range based on cancer diagnosis, a total of 11.57% of individuals tested positive, with 19.58% of them having a cancer diagnosis and 6.92% not having a cancer diagnosis.

When analyzing the rates of exceeding the reference range by the clinics that requested the test, the highest rate of 21.69% was observed in the medical oncology clinic, followed by 16.98% in the emergency medicine clinic, and 15.54% in the gastroenterology clinic. Among the clinics with the highest number of test requests in 2017-2019, the ınternal medicine clinic had a rate of 7.75%, and in 2020-2021, the medical oncology clinic had the highest rate of 21.69%, ranking first. The obstetrics and gynecology clinic, which ranked second in 2017-2019, had a rate of 6.38%. Family medicine had a rate of 4.79%.

When examining the distribution of test costs over the years, in 2017 the total cost was 39,558,952 TL with a unit cost of 19,490,305 TL, while in 2021 the total cost was 37,515,568 TL with a unit cost of 18,483,550 TL.

Discussion

Given the findings, it is evident that the CA 19-9 test is widely requested and utilized as a tumor marker. In this study, the requests and usage of the CA 19-9 test were examined in a large population sample from 2017 to 2021. The results indicate an increase in the number of tests and tests per 100,000 population over the years, with a significant decrease observed in 2020 and 2021. This provides insights into the placement and changes in the use of the CA 19-9 test in clinical practice over time.

Furthermore, the CA 19-9 test is considered a commonly used marker in the diagnosis of pancreatic cancer. Our findings demonstrate that a significant proportion of patients diagnosed with pancreatic cancer have elevated CA 19-9 levels. However, it is recommended to use the CA 19-9 test in conjunction with gold standard diagnostic methods such as pancreatic protocol CT rather than relying solely on CA 19-9 for the diagnosis of pancreatic cancer.

Additionally, CA 19-9 test can yield positive results in various conditions including benign diseases and other types of cancer. This highlights the need for caution regarding the specificity of the test. Elevated levels of CA 19-9 can reflect not only cancer but also inflammation and other benign conditions.

Lastly, our findings indicate that the CA 19-9 test impacts the rate of cancer diagnosis among patients. The results demonstrate an increasing trend in the proportion of patients receiving a cancer diagnosis over time among those who underwent CA 19-9 testing. This suggests that the test can play a significant role in cancer diagnosis and facilitate the determination of appropriate treatment regimens.

The findings of this study provide valuable insights into the role and evaluation of the utilization of the CA 19-9 test in clinical practice. However, further research and comprehensive clinical studies are warranted to better understand the accuracy, sensitivity, and specificity of the CA 19-9 test. This information can contribute to improved decision making and outcomes in the diagnosis and treatment processes for patients.

CA 19-9 is a commonly used tumor marker in the diagnosis of pancreatic ductal adenocarcinoma. Pancreatic cancer has a 5-year survival rate of less than 7% and is the fourth leading cause of cancer-related deaths. Globally, the incidence and mortality rates in 2018 were 5.5 and 5.1 per 100,000 in males and 4.0 and 3.8 per 100,000 in females, respectively (7). In Turkey, it is more frequently observed in males compared to females. Ministry of Health, Ankara Provincial Health Directorate, Turkey Cancer Statistics 2016, Ankara 2019 (8).

Every year, there is an increasing trend in the number of pancreatic cancer cases in Turkey, with a rate of 5.7 per 100,000 in males and 3.6 per 100,000 in females (9). Although pancreatic cancer is more common in males, our study found a higher proportion of CA 19-9 test requests in females. When the test counts were compared between genders, the ratio was 1.94 in 2017, 1.93 in 2018, 1.91 in 2019, 1.77 in 2020 and 1.84 in 2021.

The disease is rare in individuals under 45 years of age, with a peak occurrence in males aged 65-69 and females aged 75-79 (10).

In our study, when the test request counts were compared by age groups, the CA 19-9 test was predominantly requested in the 18-64 age group, followed by the 65 and older age groups, and the least requests were

made in the 0-17 age group. However, no analysis of age distribution by gender was conducted in our study.

Regarding the rates of exceeding the reference range based on age groups, the highest rate was observed in the 65 and older age group (16.66%), followed by the 18–64 age group (9.31%) and the 0-17 age group (8.86%).

More than 80% of patients diagnosed with pancreatic cancer had elevated CA 19-9 levels. When the rates of exceeding the reference range were examined by gender, the overall rate was 11.53%, with higher rates in males (14.87%) compared to females (9.62%). When comparing the rates of cancer diagnosis among individuals who underwent CA-19.9 testing at any time, there was an increasing trend over the years. In 2017, 28% of individuals received a cancer diagnosis, while in 2020 and 2021, the rates were 38% and 33%, respectively. In 2017, 8.43% of individuals who underwent the CA-19.9 test received a cancer diagnosis associated with CA-19.9, while 30.69% received a cancer diagnosis not associated with CA-19.9. Until 2020, these rates increased, with 16.60% of patients receiving a CA-19.9-related cancer diagnosis and 42.22% receiving a cancer diagnosis not associated with CA-19.9. In 2021, these rates were 10.76% and 39.21%, respectively.

When comparing the rates of exceeding the reference range, the highest rate was observed in 2020 (13.23%), while the lowest rate was observed in 2018 (10.39%).

When the rates of exceeding the reference range were compared by institution level, the highest rate was observed in third-level institutions, followed by second-level institutions, and the lowest rate was observed in first-level institutions.

According to the institution types, the overall rate of exceeding the reference range was 11.56%, with the highest rates observed in university hospitals, followed by private healthcare institutions, and the lowest rate observed in public hospitals.

Study Limitations

This study has several limitations. First, the data were obtained retrospectively, which means that pre-study design control could not be implemented. Additionally, there may be missing or erroneous data in the dataset. The scope of the study focused solely on the use of the CA 19-9 test and did not take into account the impact of other potential factors or variables.

Conclusion

The findings of our study suggest that the CA 19-9 test is commonly used as a marker for the diagnosis of pancreatic cancer. However, our results indicate the limitations of using the test as a standalone diagnostic tool and highlight the importance of its evaluation in conjunction with other clinical and laboratory methods. Conducting further multicenter and prospective studies will contribute to a better understanding of the accuracy and effectiveness of the CA 19-9 test.

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