

Importance of Procalcitonin Level in Serum for Postmortem Diagnosis of Sepsis

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ABSTRACT

Introduction: Sepsis is still one of the major causes of morbidity and mortality despite the improvements of diagnosis and treatment in the modern medicine nowadays. A number of studies has recently dealt with different methods and markers to better define criteria for the postmortem diagnosis of sepsis. Procalcitonin is regarded as a valuable marker for sepsis in living persons and even in post-mortem investigations. The aim of this prospective study was to investigate whether serum procalcitonin (PCT) can be used as a post-mortem marker of sepsis and to determine whether this biochemical parameter can be employed in the forensic elucidation of death due to sepsis.

Methods: 29 autopsy case with suspected common bacterial infectious diseases or sepsis were examined. As controls 27 autopsy cases were used for which there was no suspicion of a bacterial infectious disease or sepsis. Femoral or heart blood, cerebrospinal fluid(CSF), samples from lung, liver, spleen, pleural fluid and acid fluid were examined histopathologically, microbiological and biochemical for findings seen in sepsis.

Results: According to 2ng/ml cut-off level of serum PCT, there's a statistically significant difference between died with diagnosis of sepsis and with reasons of non sepsis($p<0.000$). When PCT value is ≥ 2 ng/mL was determined specificity of 72.7% and sensitivity of 79,4% for the diagnosis of sepsis.

Conclusion: This study shows that the postmortem PCT serum levels a useful marker in achieving rapid distinction between sepsis and nonsepsis-related causes of death, especially in conjunction with the medical case history and further autopsy results.

Keywords: Sepsis; Procalcitonin; Postmortem diagnosis; infection

INTRODUCTION & OBJECTIVE

Sepsis is a systemic, immunological, hormonal and metabolic response, which develops as a result of transfer of the bacteria or other pathogens to blood circulation. Despite many new treatment models, mortality rates in sepsis are currently very high due to delays in treatment.

The main differences in clinical and postmortem microbiological analysis are rather observed during the interpretation of the results of the culture. The actual discussion in this regard is on whether the isolated microorganism is the cause or result of death. Positive postmortem culture shows contamination, colonization or infection. It is important to verify whether a postmortem isolated factor is the reason of infection. Therefore macroscopic, microscopic findings, biochemical analysis and antemortem

history as well are required to support the result of culture (1). The difficulties in the diagnosis of sepsis and the lack of specific parameters has augmented the related studies. New scientific data have been obtained in recent years supporting that serum procalcitonin (PCT) level may be an important indicator for diagnosis and monitoring (2-5).

Under normal conditions, procalcitonin is a glycopeptide of 116 amino acids produced by C cells of the thyroid gland, and it is a calcitonin precursor with a molecular weight of 13 kD (3, 4). The procalcitonin level of healthy individuals is either very low (<0.15 ng/ml) or at an unidentifiable level. Serum procalcitonin level was reported to increase significantly under sepsis and serious invasive bacterial infection cases. PCT is measured over 2 ng/ml in septic circumstances, and it may increase over 100 ng/ml under serious sepsis cases.

Severe viral infections, localized bacterial infections and other non-infectious inflammatory diseases do not change the level of procalcitonin (3, 4). The studies conducted have shown that the biochemical indicators are reliable for diagnosis not only for the living sepsis cases but also for postmortem sepsis cases. There are studies which indicate that the procalcitonin level identified for the patients who died of sepsis is significantly high compared to those who are alive (3-5).

Routine histopathological studies conducted on the samples taken from the autopsies of mortal sepsis cases are not always enough for sepsis diagnosis. Publications report that sensitivity is in 80-100% and specificity is in 79-100% range for the sepsis diagnosis of PCT (4). The half-life of proinflammatory cytokines varies between minutes and a few hours, while the half-life of PCT changes between 25-30 hours. Under room temperature, the stability of PCT is much higher than those of cytokines. The concentration of PCT does not change in arterial and venous blood. Despite the inability to use hematological parameters due to postmortem haemolysis; PCT levels have maintained their stability in the studies which were frozen and saved, and then melted; and they do not represent a significant change even in the cases of haemolysis. These data provide the superiority of procalcitonin in sepsis diagnosis compared to the routinely used infection indicators such as CRP, ECR, WBC, TNF alfa (3-7).

In this study, which was conducted with postmortem cases, the procalcitonin levels in the blood samples of the case group who are considered to have died of sepsis and those of the control group cases who died of non-sepsis causes were compared. In sepsis-caused death cases, which we had difficulty in diagnosing, an agent cannot be obtained from the inspection of the culture due to intense antemortem antibiotic treatment. In addition, the sensitivity and specificity of histopathological inspections are low. Our study aimed to place procalcitonin measurement in serum into routine practice as it is a more accurate, faster and cheaper method.

TOOLS & METHOD

In this prospectively planned study, the details provided in the hospital medical records and death inspection protocols of the cases who were sent for autopsy to the Council of Forensic Medicine Morgue Department between January 2009 and February 2011, and the findings obtained during autopsy were evaluated. In

accordance with the definitions acknowledged in the ACCP/SCCM consensus conference (8); 20 male and 9 female cases suspected of sepsis as the cause of death, and 23 male and 4 female cases as the control group, who are considered to have a non-sepsis cause of death were included in the study.

Demographic data (gender, age) of the patients who were included in the study, their clinical data (date of admission and discharge, clinical diagnosis, treatment details, focus of sepsis, source of sepsis) and laboratory data (results of culture of blood and other clinical data, results of the culture of blood collected from corpses, microscopy findings, procalcitonin value, histopathological inspection results) were recorded on the monitoring form.

The cases with chronic organ failure, pregnancy, cases administered with massive blood transfusion, multiple trauma and fractures in multiple regions (due to triggering systemic inflammatory response), the cases with chronic rheumatic disease (SLE, RA, FMF, etc.) or inflammatory disease in chronic course were excluded from the study.

5 cc blood was drawn in biochemistry tubes for procalcitonin study; after resting for coagulation for 20-30 minutes, they were centrifuged at 3500 rpm for 10 minutes, and their procalcitonin levels were checked in serums stored at +4°C, with the Enzyme Linked Fluorescent Assay (ELFA) method by using VIDAS BRAHMS PCT (France) kit of Biomerieux Minividas (Italy) on the instrument of the same company.

In the studies conducted for postmortem sepsis diagnosis, the severe bacterial infection and/or sepsis probability is expressed as low at a PCT concentration under 0.5 ng/mL, and high at a PCT concentration over 0.5 ng/mL (4, 9). The concentrations under 0.5 ng/mL do not allow for the exclusion of infection due to the reasons such as localized infections without systemic symptoms or early phase (<6 hours) systemic infection. Apart from that, increased procalcitonin levels may also be identified in the absence of infection. Therefore, PCT concentration levels in the range of 0.5 and 2.0 ng/mL should be evaluated by taking into account the case's medical history. In the studies conducted on healthy men and women by using VIDAS BRAHMS PCT test, the normal values were calculated as <0.05 ng/mL and 0.09 ng/mL respectively (10, 11). These values were also considered in our study.

The analyses of the study were evaluated on SPSS (Statistical Package for Social Science) Windows 16.0 statistical software. The descriptive analyses were provided by using average and standard deviations for normally distributed variables. The normally distributed and parametric data were realized by using Independent Sample T test, and those which do not show normal distribution and non-parametric data were realized by using Chi-square test. Comparison of multiple data has been realized by using one way analysis of variance (ANOVA), and comparative analyses were carried out through ROC analysis in the identification of cut-off value for sensitivity and specificity values. The cases with a P value under 0.05 were considered as statistically significant.

FINDINGS

Epidemiological Findings

The data of 56 autopsied cases were evaluated in our study. The distribution of all cases according to the gender and percentage showed 43 male (76.8%) and 13 female (23.2%) cases. Respectively 20 male (69.0%) and 9 female (31.0%) cases were observed in the case group suspected of sepsis, and 23 male (85.2%) and 4 female (14.8%) cases in the control group.

According to the information obtained from the cases' postmortem examination report, the group suspected of sepsis includes 29 cases (51.8%) and the control group includes 27 cases (48.2%). 26 cases suspected of sepsis (89.7%) died when they were hospitalized as inpatients, 3 cases (10.3%) were found death at home or they were found death outside and brought for autopsy. In the control group, 19 cases (70.4%) were brought for autopsy after being found death at home or outside, 8 cases (29.6%) were brought to hospital and died before completing 24 hours.

The average hospitalization period of all cases was calculated as 11.3 ± 20.8 days; that of sepsis group cases as 21.59 ± 24.9 days, and that of control group cases as 0.4 ± 2.3 .

It was identified that 20 cases (69.0%) from the sepsis group received infection diagnosis and/or treatment before death, 5 cases (17.2%) did not receive infection diagnosis and/or treatment, and 4 cases (13.8%) were unknown whether they received infection diagnosis and treatment. It was identified that 1 case from the control group (3.7%) received infection diagnosis and/or treatment, 25 cases (92.6%) did not receive infection diagnosis and/or treatment, and

whether 1 case (3.7%) received infection diagnosis and treatment was unknown.

Autopsy Findings

Upon the inspection of the distribution of the organs which were macroscopically considered to have infection during the autopsy of sepsis cases; macroscopic infection was identified in lungs of 11 cases (37.9%), in multiple organs of 5 cases (17.2%), in the digestive system of 1 case (3.4%), in the skin-muscle structure of 1 case (3.4%), and any infection findings were not identified macroscopically on 11 cases (37.9%). In the control group cases, any infection findings were not identified macroscopically on 24 cases (88.9%), and infection in lung was identified in 3 cases (11.1%).

Histopathological Findings

According to the infection finding detection rate in the histopathological inspections of sepsis group cases; signs of infection were detected on 18 cases (62.1%), and not detected on 11 cases (37.9%). As a result of the inspection, notably lung pathologies constitute the majority. Mostly pneumonia, bronchitis, pleuritis and bronchopneumonia were identified in relation with lungs. Histopathological inspections of the control group cases showed signs of infection on 7 cases (25.9%), and did not show on 20 cases (74.1%). Lung pathologies were identified most also on these 7 cases identified with signs of infection.

Microbiological Findings

In the sepsis group, reproduction was identified in the aerobic blood culture of 20 cases (69.0%), CSF culture of 19 cases (65.5%), lung culture of 15 cases (51.7%), and spleen culture of 7 cases (24.1%). Reproduction was observed on all of 13 cases from whom pleural liquid was taken, and on all of the 6 cases from whom abdomen liquid was taken.

Upon the classification of the bacteria that reproduce in blood in the sepsis group, among the 29 cases with blood culture for Gram staining, 16 (55%) indicated Gram negative bacteria growth, 2 (6.9%) Gram positive bacteria, 2 (6.9%) both Gram negative and Gram positive bacteria; and no growth in 9 cases (31.0%). It was identified that the Gram staining specifications of the bacteria grown in the blood culture do not have a statistically significant effect on serum PCT ($P=0.463$).

Forensic Medicine Findings

The classification of the causes of death of our cases in the sepsis group showed that 21 cases (72.4%) died due to sepsis, septic shock or severe bacterial infection; 8 cases (27.6%) due to non-sepsis causes, 7 cases (87.5%) due to trauma and developed complications, and 1

(12.5%) case due to cancer and developed complications (Table 1). Among the control group cases, 26 cases (96.3%) died due to non-sepsis causes, 1 case (3.7%) due to lung infection and developed complications, and the procalcitonin level was identified as 1.27 ng/ml.

Table1. Patients' characteristics of the sepsis disease group

Patient No	Cause of death at autopsy	PCT (ng/ml)	Clinical diagnosis/treatment of infection	Hospital admission	Histopathological findings	Post-mortem blood culture
1	Sepsis due to pneumonia and meningitis	5,01	Yes	Yes	pyemik abscess foci on lung and brain; purulent bronchitis	No
2	Goodpasture's syndrome and complications	6,39	Yes	Yes	Kidney: abundant eosinophilic homogeneous material assets in tubulus lumen (fibrin?)	No
3	Burn with SIRS and clinically diagnosed beginning sepsis	19,58	Yes	Yes	Skin: Focal loss of the epidermis, the dermis hemorrhage, perivascular mononuclear inflammatory infiltration	<i>Escherichia coli</i>
4	Sepsis due to developing pneumonia after URTI*	6,22	Yes	Yes	The presence of a small number of hemosiderin-laden macrophages in the alveolar lumen	<i>Proteus spp.</i>
5	Peritonitis, pneumonia	28,12	Yes	Yes	fresh lobular pneumonia, purulent bronchitis, fibrin purulent peritonitis on omentum	<i>Klebsiella spp.</i>
6	Pneumonia	0,61	Yes	Yes	purulent bronchitis, fresh lobular pneumonia	<i>Klebsiella spp./Escherichia coli</i>
7	Cerebral hemorrhage	1,27	Yes	Yes	intracerebral hemorrhage of the brain, the bleeding in dura mater	Mixture**
8	Pneumonia	16,87	Yes	Yes	abscess lobular pneumonia, purulent fibrinous pleuritis	No
9	Sepsis due to peritonitis after gastric operation and pneumonia	37,04	Yes	Yes	organized lobular pneumonia, purulent bronchitis, organized fibrinous pleuritis, peritonitis organized fibrin in the mesentery	<i>Klebsiella spp./Escherichia coli</i>
10	Pneumonia	5,94	Yes	Yes	organized lobular pneumonia	<i>Escherichia coli</i>
11	Burn and its complications, pneumonia	10,37	Yes	Yes	lobular pneumonia, purulent bronchitis, acute inflammation of the skin ulcer	<i>Enterobacter spp.</i>
12	Cerebral hemorrhage, brain tissue damage	0,46	Yes	Yes	Brain: Subarachnoid hemorrhage, intraparenchymal hemorrhage areas	<i>Enterococcus spp.</i>
13	Sepsis due to peritonitis	2,06	Yes	Yes	peritonitis showing the organization in small and large intestines	No
14	pulmonary embolism	4,95	Yes	Yes		<i>Klebsiella spp.</i>
15	extensive soft tissue hemorrhage and complications	25,43	Yes	Yes		No
16	sepsis due to small intestine inflammation	1,79	Yes	Yes	partly organized acute inflammation of the small intestine	<i>Klebsiella spp.</i>
17	Larynx Carcinoma and related complications	0,74	Unknown	No	larynx; moderately differentiated squamous cell carcinoma,	<i>Escherichia coli/ Klebsiella spp.</i>
18	diffuse brain hemorrhage	0,05	No	Yes	Brain: diffuse subarachnoid hemorrhage,	<i>Pseudomonas spp./ Klebsiella spp.</i>
19	Acute cardiac failure with bronchitis	0,71	Unknown	Yes	Lung: Polymorphonuclear neutrophils in interstitial capillary lumen (inflammatory stasis)	<i>Pseudomonas aeruginosa</i>
20	Sepsis due to peritonitis due to intestinal perforation	15,96	No	Yes	Purulent inflammation of the spleen, partially organized fibrin purulent peritonitis	Gram-negative enteric bacilli
21	Sepsis due to meningitis	4,8	Yes	Yes	polymorphonuclear leukocyte infiltration of rich acute inflammation in the M.spinalis, acute bacterial endocarditis on mitral valve	<i>Escherichia coli</i>
22	Pneumonia	2,68	Unknown	Yes	abscess lobular pneumonia, purulent bronchitis	<i>Enterococcus spp./ Staphylococcus aureus</i>
23	Sepsis due to aspiration pneumonia	0,76	No	Yes	fresh lobular pneumonia, purulent bronchitis, purulent pleuritis	No
24	Congestive heart failure and sepsis due to pleuritis	24,38	No	Yes	purulent fibrinous pleuritis, The subdural fibrin exudation in M.spinalis' and PNL	<i>Staphylococcus aureus/ Escherichia coli</i>
25	Sepsis, developing a systemic infection of the vagina and rectum tear	1,91	No	Yes	vagina and rectum, partly organized mixed inflammatory cell infiltration	<i>Pseudomonas spp</i>
26	Sepsis due to pneumonia	45,88	Yes	Yes	fresh lobular pneumonia, purulent bronchitis, acute hemorrhagic pancreatitis	<i>Acinetobacter baumannii/ Citrobacter freundii</i>
27	Intraabdominal sepsis after intestinal operation	1,89	Yes	Yes	purulent fibrinous inflammation in omentum	No
28	Sepsis with status post shoulder replacement	200	Yes	Yes		No
29	Pneumonia	11	Unknown	No	Acute bronchitis, bronchopneumonia	No

Comparative analyses

The comparison of the procalcitonin levels of our cases suspected of sepsis and those of our control group showed the average procalcitonin level of 29 cases suspected of sepsis as 16.6 ± 37.2 , and that of the 27 cases in the control group as 1.11 ± 1.3 . The difference between the averages of serum procalcitonin levels between the group diagnosed with sepsis and the control group was found statistically significant ($P=0.033$).

During the histopathological inspection, the comparison of the existence of the infection finding with the 2 ng/ml cut-off value of procalcitonin showed that PCT remained under 2 ng/ml at 24 of the 32 cases without infection finding, and over 2 ng/mg at 8 cases; while PCT remained under 2 ng/m at 9 of the 24 cases, and over 2 ng/ml at 15 cases. The difference between the infection findings identified in histopathological inspection according to serum PCT cut-off value was found statistically significant ($P=0.005$).

Upon comparing the autopsy report results of our cases with non-sepsis causes of death to sepsis, septic shock or severe bacterial infection cause of death according to 2 ng/ml Cut-off value; 27 of the cases who were identified as died of non-sepsis reasons had a PCT value under the cut-off value, and 7 over the cut-off value. Meanwhile, 6 of the cases with sepsis, septic shock or severe bacterial infection had a PCT value under the cut-off value, and 16 over the cut-off value (Table 2). The difference in terms of serum PCT cut-off values between the cases with non-sepsis causes of death and those with sepsis, septic shock or severe bacterial infection cause of death was found statistically significant ($P=0.000$). Upon considering the causes of death of our cases and serum procalcitonin levels together, the sensitivity and specificity of the 2 ng/ml cut-off value chosen to set postmortem diagnosis of sepsis were calculated as 72.7% and 79.4%, respectively (Table 3).

Table2. Comparison of the Final Report Results with procalcitonin cut-off value

PCT Cut-off Value	Sepsis group	Non-Sepsis group	Total
≥ 2 ng/ml	16	7	23
< 2 ng/ml	6	27	33
Total	22	34	56

Table 3. Calculation of the sensitivity and specificity (cut-off: 2 ng/ml)			
Result	Sepsis	Non-Sepsis Caused	Total
≥ 2 ng/ml	16	7	23
< 2 ng/ml	6	27	33
	22	34	56
Sensitivity 72,7 %		Likelihood ratio for positive test results:	3,5
Specificity:79,4 %		Likelihood ratio for negative test results:	0,34
Prevalence (Pre test probability) :%39,2		Pre-Test Odds:	0,64
Positive predictive value %69,5		Post-Test Odds:	2,28
Negative predictive value: %81,8		Post-Test probability: %69	

Considering the data in our study, we conducted a ROC analysis to calculate our PCT cut-off value, and the cut-off value for our cases has been identified as 1.57 ng/ml. The area under ROC curve is 0.86, and it was been identified to have a high reliability as a variable which can be used for sepsis diagnosis of the calculated value (Figure 1). According to these data, the

sensitivity and specificity of the 1.57 ng/ml cut-off value were calculated as 81.8% and 79.4%, respectively. The sensitivity and specificity according to this new value are provided on the table. The sensitivity rate -in other words the ratio of correct diagnosis of the cases which are actually sepsis- increased with the changed cut-off value, (Table 4).

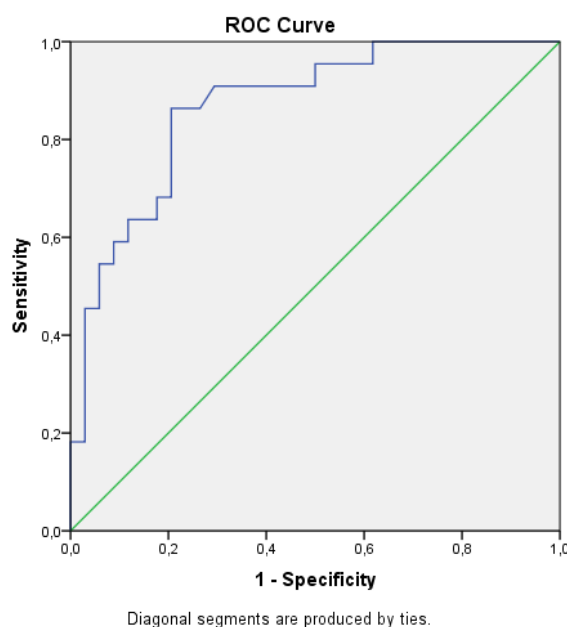


Figure1. ROC curve of PCT analysis in postmortem sepsis diagnosis (AUC is 0.868 (95% CI: 0.774-0.962))

Table 4. Calculation of the sensitivity and specificity (cut-off: 1.57 ng/ml)			
Cut-off PCT Value	Sepsis	NonSepsis Caused	Total
≥ 1.57 ng/ml	18	7	25
< 1.57 ng/ml	4	27	31
Total	22	34	56
Sensitivity :	81,8 %	Likelihood ratio for positive test results: 3,97	
Specificity :	79,4 %	Likelihood ratio for negative test results: 0,22	
Prevalence (Pre test probability):	39,2 %	Pre-Test Odds:	0,64
Positive predictive value	72%	Post-Test Odds:	2,57
Negative predictive value:	87%	Post-Test probability:	72%

DISCUSSION AND CONCLUSION

Sepsis is a table which holds a prominent place in forensic medicine practice due to its high mortality. Postmortem diagnosis of the cases considered to have died of sepsis is very difficult due to non-specific histopathological and microbiological findings. Results of culture, the gold standard in the diagnosis of sepsis, cause debate due to postmortem changes. Therefore, the result of culture must be supported with macroscopic, microscopic findings, biochemical and serological analysis as well as the antemortem history (1).

In a study, the PCT level of the cases who died of sepsis was identified higher compared to the living group, and it was concluded that procalcitonin was a reliable indicator for the identification of prognosis (12). In another study on sepsis patients to identify the value of PCT both as an inflammatory parameter and an indicator of prognosis, sepsis patients were divided into two groups as living and deceased patients, and PCT levels of the deceased patients

were found evidently higher (13). In our study, the antemortem data of our control group, who were thought to have died of sepsis and that of our control group who were thought to have died of non-sepsis causes, their autopsy findings, histopathological and microbiological findings and serum PCT levels were compared. As a result, whether PCT, which is commonly used in clinics, was an indicator that could comfortably be used also for postmortem applications was examined.

The studies showed that serum PCT half-life is 25-30 hours at in vitro settings, it maintains its stability up to postmortem 140 hours, and there is not an evident difference between the measured antemortem and postmortem values (3, 4, 9). PCT's postmortem cut-off value in the diagnosis of severe bacterial infections and sepsis varies among studies. Many researchers have accepted 2 ng/ml as the cut-off value in postmortem sepsis diagnosis, while some authors have considered the cut-off value as 1.5 or 1.1 ng/ml for burnt and trauma patients (4, 9, 11, 14, 15-17). In our study, 2 ng/ml cut-off

value was taken into consideration and the comparative analyses were realized accordingly (4, 9).

Similar to the studies of Tsokos, Bode-Janisch and Ramsthaler *et al.*, the highest histopathological finding was identified in lung also in our study; and the most frequent infection focus was identified as the respiratory system (4, 9, 18). Similar results were obtained also in the clinical studies conducted by Brun-Buisson and Sand *et al.* and the most frequent focus was identified as the respiratory system (19, 20). However, it is claimed that a significant difference does not occur between serum PCT level and the affected organ or the degree of spread. In the studies conducted by Bode-Janisch and Tsokos *et al.*, histopathological inspections are considered as inevitable in setting sepsis diagnosis, while it is claimed that they are not at a level of sufficient sensitivity and specificity to distinguish an ordinary infection table from sepsis (3, 18). It was indicated only in the publications of Tsokos *et al.* that granulocytic infiltration which may be observed in the portal tract is relatively more specific in sepsis (3). In our study, the relation between the identification of infection during histopathological inspection and sepsis diagnosis was compared and found statistically significant in identifying the result of histopathological inspection ($P=0,000$). However, in the conducted inspection, it was identified that the diagnoses were not sepsis-specific findings, but they were the infection tables which could also be seen without the development of sepsis syndrome, such as fresh lobular pneumonia, bronchopneumonia, pleuritis, pyogenic bronchitis, peritonitis, focal abscesses, and inflammatory cell infiltrations. As a result, we reached the conclusion that histopathological inspection is essential for sepsis diagnosis, but it is not a sufficient entity on its own.

Histopathological data of both groups in our study were compared according to PCT 2 ng/ml cut-off value, and the ratio of detecting infection signs histopathologically was found statistically highly significant ($P=0,000$). The probability to identify signs of infection in histopathological examination increases when the value of procalcitonin is over 2 ng/ml according to our study.

The difference between antemortem and postmortem cultures was noted in various publications, and the microorganisms which are

not actually active were produced in the post-mortem collected cultures due to rapid bacterial invasion (16, 21-23). In the study conducted by Reznicek and his colleagues, the same organism was produced in the postmortem blood culture of only 34% of the known cases with antemortem bacteriemia/fungemia diagnosis. 76% of the isolated factors were evaluated as contaminant reproduction (24). Wrong positive or wrong negative result potential is higher in lung cultures compared to other organ cultures (25-27). In a study conducted by Dolan *et al.*, although any proven causes of death associated with infection were not found in 211 tissue samples taken from randomly selected 67 autopsies, reproduction was observed on 17 (68%) of 25 lung cultures. Reproduction was identified in 19 cases (73%) among 26 lung cultures which are known to be infectious (28). As will be understood, high contamination rate is the most important factor which limits diagnostic use of postmortem cultures. Caplan *et al.* emphasize that polymicrobial reproduction is most frequently observed in lungs, the diagnosis of the infection requires the inflammatory response of host together with the invasion of the pathogenetic microorganism to tissue or body liquid in general; therefore, observation of leukocyte in Gram staining becomes more significant (29, 30).

Tsokos and Ramsteler *et al.* claim that serum procalcitonin levels at this stage are a highly effective biochemical indicator to separate agent/contamination (3, 4, 27).

It is reported that in the past years, Gram positive bacteria appeared before us more frequently as the reason of sepsis; while Gram negative bacteria have been on the forefront in the studies conducted during the last 20 years (31). In the study conducted by Ballester *et al.* from 1995 to 2004 including 33,767 patients; most frequently Gram negative bacterial were noted etiologically among all sepsis cases (21.4%) and it was followed by Gram positive bacteria (17%) and fungi (3.1%) (32). In the study of Meriç *et al.* in our country, the most frequently reproduced microorganisms in blood culture were identified as *S.aureus*, *Acinetobacter spp*, *P. aeruginosa*, *Enterococcus spp*, *E.coli*, *Candida spp*, *K. pneumonia*, *KNS* and *Enterobacter spp*, respectively (33). Gram negative bacteria (55%) were identified at the first rank on looking at the distribution of active microorganisms in our study. Gram positive bacteria (6.9%) and polymicrobial (6.9%) reproductions were noted in less frequency.

Upon the inspection of the most reproduced microorganisms in blood culture; mostly *Klebsiella spp*, *E. coli* and *Pseudomonas spp*. reproduced among Gram negatives, while Enterococs and *S. aureus* reproduced among gram positives. These identifications support the data from the other studies.

Many clinic and postmortem studies were conducted to evaluate diagnostic performance of PCT in sepsis and septic shock. Results of most of these studies support the opinion that PCT is a bioindicator which can be used in setting sepsis diagnosis. In the study conducted by Endo *et al.* including 82 patients in 7 hospitals, it was found that 9 patients satisfied SIRS criteria while 20 patients did not satisfy SIRS criteria, 34 patients were diagnosed with sepsis and 19 patients with serious sepsis, median PCT level of the patients in serious sepsis group was 36,1 ng/ml and significantly higher ($P < 0,01$) compared to the sepsis group (0,6 ng/ml) (34). In our study, upon the comparison of the averages of serum procalcitonin levels of the suspected case group and the control group; the difference between the averages of serum procalcitonin levels was found statistically significant ($P=0,035$).

Upon the examination of the autopsy report results of our cases, independent from the work groups we separated at the beginning, totally 56 cases were divided into two groups as those died of sepsis and those died of non-sepsis causes. Serum PCT values which are identified by taking into account the final results were compared with 2 ng/ml cut-off values and the difference between the two groups was statistically found significant at an advanced level ($P=0,000$), and biochemical indicator was a valid indicator also for postmortem sepsis diagnosis similar to clinical diagnosis.

In the study of Brunkhorst *et al.*, for 2 ng/mL and higher values of PCT, sepsis and septic shock diagnosis sensitivity and specificity were reported as 96% and 86% (35). In the research conducted by Ramsthaler *et al.*, sensitivity and specificity in postmortem sepsis diagnosis were respectively found as 86.9% and 94.4% according to 2 ng/ml cut-off value, and as 95.6% and 63.5% according to 0.5 ng/ml cut-off value (4). In our study, upon considering the causes of death of our cases and serum procalcitonin levels together, the sensitivity and specificity of the 2 ng/ml cut-off value chosen to set postmortem diagnosis of sepsis were calculated as 72.7% and 79.4%, respectively.

Considering the autopsy results of our cases and PCT levels, the cut-off value was calculated as 1.57 ng/ml as a result of the conducted linear regression analysis and ROC analysis; and sensitivity and specificity were respectively identified as 81.8% and 79.4% according to this new value (Table 4). The values given on the table showed that we could achieve more correct results with the 1.57 ng/ml cut-off value which we identified. In the study conducted by Lavrentieva *et al.* in the literature, the sensitivity and specificity for diagnosis performance of sepsis cases were respectively found as 88.3% and 92.3% when PCT cut-off value is considered as 1.5 ng/ml (14).

It is continuously emphasized in the literature that postmortem microbiological analyses should be evaluated with clinical, autopsy and histopathological findings (25, 36, 37). "Pathological-microbiological compliance" and "clinical-pathological-microbiological" compliance concepts are mentioned. "Pathological-microbiological compliance" is defined as an independent compliance between postmortem culture results and autopsy results independent from the existence of an infectious disease, while "clinical-pathological-microbiological" compliance is defined as the existence of a clinical history which supports sepsis or localized infection in addition to pathological-microbiological compliance (37). The compliance between pathology and microbiology has an important impact on the interpretation of the results of the culture. This study and the studies on postmortem sepsis diagnosis in the literature point that biochemical compliance to be identified with serum PCT measurement will have an impact to increase the verification rate in the interpretation of the results. The ideal scenario is the identification of serum PCT level in biochemical analyses together with obtaining the microorganism in correlation with the clinical diagnosis and the macroscopic and microscopic findings of autopsy, thus assuring full clinical-pathological-microbiological-biochemical compliance (3, 4, 38, 39). As a result, serum PCT level is an important, reliable, repeatable and fast indicator in setting postmortem diagnosis of sepsis.

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