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An immunohistochemical study of the diagnostic value of TREM-1 as marker for fatal sepsis cases

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ABSTRACT

Triggering receptor expressed on myeloid cells-1 (TREM-1) is produced and up-regulated by exposure of myeloid cells to lipopolysaccharides or other components of either bacterial or fungal origin, which causes it to be strongly expressed on phagocytes that accumulate in inflamed areas. Because TREM-1 participates in septic shock and in amplifying the inflammatory response to bacterial and fungal infections, we believe it could be an immunohistochemical marker for postmortem diagnosis of sepsis. We tested the anti-TREM-1 antibody in 28 cases of death by septic shock and divided them into two groups. The diagnosis was made according to the criteria of the Surviving Sepsis Campaign. In all cases, blood cultures were positive. The first group was comprised subjects that presented high ante-mortem serum procalcitonin and the soluble form of TREM-1 (s-TREM-1) values. The second group comprised subjects in which s-TREM-1 was not measured ante-mortem. We used samples of brain, heart, lung, liver and kidney for each case to test the anti-TREM-1 antibody. A semiquantitative evaluation of the immunohistochemical findings was made. In lung samples, we found immunostaining in the cells of the monocyte line in 24 of 28 cases, which suggests that TREM-1 is produced principally by cells of the monocyte line. In liver tissue, we found low TREM-staining in the hepatocyte cytoplasm, duct epithelium, the portal-biliary space and blood vessel. In kidney tissue samples, we found the TREM-1 antibody immunostaining in glomeruli and renal tubules. We also found TREM-1 staining in the lumen of blood vessels. Immunohistochemical staining using the anti-TREM-1 antibody can be useful for postmortem diagnosis of sepsis.

KEYWORDS

Immunohistochemistry; marker; postmortem diagnosis; sepsis; septic myocardial dysfunction; septic shock; triggering receptor expressed on myeloid cells-1

Triggering receptor expressed on myeloid cells-1 (TREM-1) belongs to the immunoglobulin superfamily (Barraud and Gibot 2011). It is expressed on neutrophils and mature monocytes, and the genes that code it are grouped on human chromosome 6. TREM-1 is expressed also on nonmyeloid cells, such as epithelial or endothelial cells (Bouchon et al. 2000). TREM-1 is produced and up-regulated by exposure of myeloid cells to lipopolysaccharides or other microbial components of either bacterial or fungal origin, which causes it to be strongly expressed on phagocytes that accumulate in inflamed areas (Bleharski et al. 2003; Yuan et al. 2012).

TREM-1 has an alternatively spliced variant that lacks the transmembrane region, which results in secretion of the receptor in a soluble form (s-TREM-1) (Weiss et al. 2017). s-TREM-1 is a 17-kDa fragment that is detectable in body fluids such as blood, broncho-alveolar lavage and cerebrospinal fluid. This molecule can be dosed in biological fluids (Brenner et al. 2016). Plasma levels of s-TREM-1 > 60 ng/ml seem to be more accurate than other clinical or laboratory tests for identifying patients with an ongoing infection (Giamarellos-Bourboulis et al. 2006). s-TREM-1 is released by activated phagocytes and can be found in plasma, pleural fluid, cerebrospinal liquor and urine (Arízaga-Ballesterosa et al. 2015; Latour-Pérez et al. 2010; Wu et al. 2012).

Combined with other markers, s-TREM-1 appears to be valuable for differential diagnosis of a non-infective sepsis with systemic inflammatory response syndrome (SIRS) (Bouchon et al. 2001; Oku et al. 2013). In the forensic field, reliable diagnosis of sepsis may be even more difficult owing to unavailable medical records at autopsy, nonspecific autopsy and histological findings including myocardial ischemia, pulmonary edema, hypoxic liver damage, mesenteric ischemia, gastrointestinal hemorrhage, spleen infarction, kidney ischemia and brain edema that may have either an infectious or non-infectious origin.

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Because of the crucial role that TREM-1 plays in increasing septic shock and amplifying the inflammatory response to bacterial and fungal infections, we suspect that it could be a useful immunohistochemical marker for postmortem diagnosis of sepsis.

Material and methods

Our data are covered by the general authorization to process personal data for scientific research purposes granted by the Italian Data Protection Authority (1 March 2012 as published in Italy's Official Journal no. 72 dated March 26 2012), because the data do not include significant personal information concerning the subjects. Our study does not involve the application of experimental protocols; therefore, it does not require approval by institutional and/or licensing committees. In all cases, local prosecutors opened an investigation and ordered that an autopsy be performed to clarify the exact cause of death. We selected seven women and 21 men, mean age 58.2 years, who died from septic shock with an ante-mortem diagnosis of sepsis. The diagnosis was made according to the criteria of the Surviving Sepsis Campaign (Singer et al. 2016). Blood cultures were positive in all cases. We subdivided the cases into two groups. The first group comprised 14 subjects that presented high ante-mortem serum procalcitonin and s-TREM-1 values. The diagnostic evaluation of s-TREM-1 was performed using receiver operating characteristic (ROC) analysis. Areas under the curve (AUCs) were calculated. The second group comprised 14 subjects in which s-TREM-1 was not measured ante-mortem. Data concerning the subjects are given in Table 1.

Table 1. Ante-mortem serum and main autopsy and histology findings.

Case	PCT ante-mortem	s-TREM-1 ante-mortem	Ante-mortem microbiology in blood	Main autopsy and histology findings
1	29.8	120.0	*Acinetobacter baumannii + S. aureus*	*Pneumonia*
2	17.34	80.4	*E. coli*	*Peritonitis*
3	16.22	64.15	*K. pneumoniae*	*Pneumonia*
4	37.04	100.74	*S. aureus*	*Necrotizing fasciitis + pneumonia*
5	25.15	75.20	*E. faecalis + E. coli*	*Peritonitis*
6	20.15	88.50	*St.epidermidis + S. aureus*	*Pneumonia-hip prosthesis infection*
7	33.08	93.13	*E. faecalis + E. coli*	*Abdominal and pelvic infection*
8	28.09	97.90	*E. coli + P. aeruginosa*	*Pneumonia + urinary infection*
9	18.64	68.75	*Acinetobacter baumannii*	*Pneumonia*
10	30.44	98.00	*K. pneumoniae*	*Pneumonia*
11	16.44	66.88	*S. aureus*	*Pneumonia*
12	32.42	90.26	*P. aeruginosa*	*Pneumonia*
13	18.64	86.68	*E. coli*	*Peritonitis*
14	34.76	98.24	*K. pneumoniae*	*Pneumonia*
15			*K. pneumoniae*	*Pneumonia*
16			*P. aeruginosa*	*Pneumonia*
17			*E. coli + K. pneumoniae*	*Abdominal and pelvic infection*
18			*P. aeruginosa*	*Necrotizing fasciitis*
19			*L. monocytogenes + K. Pneumoniae*	*Pneumonia*
20			*Stenotrophomonas maltophilia + P. aeruginosa*	*Pneumonia*
21			*P. aeruginosa*	*Pneumonia*
22			*P. aeruginosa + S. Aureus*	*Pneumonia + peritonitis*
23			*Acinetobacter baumanii*	*Pneumonia*
24			*E. Coli*	*Peritonitis*
25			*Acinetobacter baumanii*	*Pneumonia*
26			*K. pneumoniae*	*Pneumonia*
27			*L. Monocytogenes*	*Pneumonia*
28			*E. Coli*	*Peritonitis*

We chose non-infective cases as a control group of adults (n = 14; six women, eight men, mean age 49.4 years) with no clinical history or macroscopic or microscopic signs of infection whose deaths were characterized by suddenness. We tested the anti-TREM-1 antibody on subject that died from opioid overdose (n = 5), hanging (n = 4), car accident (n = 4) and sudden cardiac death (n = 1). The postmortem interval was ≤ 36 h in each case. We used five samples, brain, heart, lung, liver and kidney for each case.

Samples, 8 cm^2, of each organ from each case were fixed in 10% buffered formalin, then washed with phosphate-buffered saline (PBS) and subsequent dehydration was carried out using a graded alcohol series. After dehydration, samples were cleared in xylene, and embedded in paraffin. Sections were cut at 4 μm, mounted on slides and covered with 3-amminopropyltriethoxysilane (Fluka, Buchs, Switzerland). A routine microscopic histopathological study was performed after hematoxylin and eosin (H & E) staining (Fischer et al. 2008).

To test the anti-TREM-1 antibody (clone L5-B8.2A12.3A12; Novusbio, Abingdon, UK), we used a biopsy sample of stomach wall from a living patient with chronic gastritis caused by a *Helicobacter pylori* infection. The biopsy sample was fixed in 10% buffered formalin, then washed with PBS and subsequent dehydration was carried out using a graded alcohol series. After dehydration, samples were cleared in xylene and embedded in paraffin. Sections were cut at 4 μm, mounted on slides and covered with 3-amminopropyltriethoxysilane (Fluka, Buchs, Switzerland). The sections were re-hydrated through graded alcohols and incubated for 20 min in methanol containing 10% H_2O_2 to block endogenous peroxidases. The sections were pretreated with citrate buffer (10 mM sodium citrate, pH 6.0), incubated with the anti-TREM-1 antibody (clone L5-B8.2A12.3A12; Novusbio, Abingdon, UK) diluted 1:500, then treated with BrightVision®

PolyHRP-anti rabbit (Vector Labs, Peterborough, UK) and developed using 1% H_2O_2 and DAB (Sigma-Aldrich, Darmstadt, Germany) in 0.05 M Tris-HCl (pH 7.9) (Yuan et al. 2014). The anti-TREM-1 exhibited staining in the stomach epithelium and glands, which served as a positive control. All postmortem samples from brain, heart, lung, liver and kidney, were immunostained using the method described above for the stomach wall biopsy.

For quantitative analysis, in each immunohistochemical section we made 20 observations in different fields/slide. The TREM-1 stained cells were counted at 40 x using a light microscope coupled to a high resolution color video camera. The number of stained cells was divided by the total number of cells and multiplied x100. The immunohistochemical positivity score was defined as: 0, nuclear and/or cytoplasm staining absent; 1, nuclear and/or cytoplasmic staining, 25%; 2, nuclear and/or cytoplasmic staining, 50%; 3, nuclear and/or cytoplasmic staining, 75%; 4, nuclear and/or cytoplasm staining, 100%. We also quantified immunohistochemical staining in blood vessel as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable, 100%. A subjective scoring system for the staining intensity of cells was defined. Five degrees of intensity were defined as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable, 100%). The most intense coloration, grade 5, was that of the stomach wall sample with chronic gastritis that was used as the positive control. In cases of heterogeneous intensity, we considered the prevailing intensity. All evaluations were performed in a double blind manner. In cases of divergent scoring, a third observer decided the final score.

Results

Our findings are summarized in Tables 2 and 3. We found blood vessel immunostaining in kidney tissue samples in 25 cases (Figure 1a). In lung, we found clear staining in lumen of the blood vessels and cytoplasm of myelomonocytes in 24 of 28 cases (Figure 1b). In myocardial samples, we found clear immunostaining in blood vessels and no staining of the cytoplasm or nucleus of myocardial cells (Figure 1c). No anti-TREM-1 staining was found in any brain samples or nuclei of nerve cells; we found blood vessel staining in all cases (Figure 1d). In lung samples we found immunostaining in the cells of the monocyte line in 24 of 28 cases, which suggests that TREM-1 might be produced principally by cells of the monocyte line (Figure 2a). We found little staining in hepatocytes of the liver in 21 of 28 cases (Figure 2b). In 14 cases, immunostaining appeared in the duct epithelium or in the portal-biliary space, and in 18 cases we found immunostaining in the blood vessels. In 25 of 28 kidney tissue samples, we found immunostaining in the glomeruli (Figure 3a), in 20 cases we found staining in the renal tubules and in 25 cases we found staining of blood vessels (Figure 3b).

Our findings for the two groups were similar, but the first group showed a greater immunohistochemical staining and intensity. The anti-TREM-1 antibody exhibited no reaction in organs or blood vessels of cadavers who died from non-infective causes (control group).

Finally, we detected greater TREM1 staining in the lumen of the blood vessels of first group with documented ante-mortem s-TREM-1 concentrations (Figure 4a–d).

Discussion

We used an experimental model and immunohistochemistry to conduct an investigation of postmortem TREM-1 expression. Various immunohistochemical markers have been used to investigate cases of sepsis, especially with regard to the lung, including markers for E-selectin, very late antigen 4 (VLA-4), intracellular adhesion molecule 1 (ICAM-1) and lactoferrin (Müller et al. 2008; Herwig et al. 2013; Galassi et al. 2018).

Tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) are found intermittently or briefly during an inflammatory response and during sepsis (Carsin et al. 1997). We reported earlier that anti-procalcitonin antibody can be a valuable tool for postmortem diagnosis of sepsis (Maiese et al. 2017). Nevertheless, definitive techniques that can be used to make a postmortem diagnosis of sepsis are lacking. Macroscopic and microscopic pathological findings are nonspecific and may be inadequate to render a definitive diagnosis for forensic purposes when clinical documentation is lacking (Tsokos 2007).

Gibot et al. (2004) reported the concentrations of C-reactive protein (CRP), procalcitonin (PCT) and TREM-1 in the plasma of 76 patients in intensive care units. These markers were stronger in infected patients than in patients who did not present with SIRS. The plasma soluble TREM-1 levels had the greatest discriminative value, with an area under the receiver-operating characteristic curve of 0.97, followed by procalcitonin (area under the curve = 0.85) and C-reactive protein (area under the curve = 0.77). At a cutoff level of 60 ng/ml (ELISA Human TREM-1 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN; sensitivity: 15.2 pg/ml), soluble TREM-1 yielded a sensitivity of 96%, a specificity of 89%, a positive predictive value of 94% and a negative predictive value of 93% using StatView software (Abacus Concepts, Berkeley, CA) for

Table 2. Qualitative and quantitative results for immunohistochemical staining for TREM 1 and ante-mortem s-TREM values: group 1.

Case	Brain	Heart	Lung	Liver	Kidney
1	Quantitative 0; V 4 Qualitative 3	Quantitative 0; V 3 Qualitative 3	Quantitative macrophages 4(c); V 4 Qualitative 4	Quantitative hepatocytes 3 (c) Bile ducts 4; V 3 Qualitative 3	Quantitative glomeruli 4 tubules 3; V 4 Qualitative 3
2	Quantitative 0; V 1 Qualitative 1	Quantitative 0; V 2 Qualitative 2	Quantitative macrophages 3(c); V 2 Qualitative 2	Quantitative hepatocytes 2 (c) Bile ducts 2; V 1 Qualitative 2	Quantitative glomeruli 2 tubules 1; V 2 Qualitative 1
3	Quantitative 0; V 1 Qualitative 1	Quantitative 0; V 1 Qualitative 1	Quantitative macrophages 1(c); V 2 Qualitative 2	Quantitative 0; V 0 Qualitative 0	Quantitative glomeruli 1 tubules 0; V 1 Qualitative 1
4	Quantitative 0; V 3 Qualitative 3	Quantitative 0; V 3 Qualitative 4	Quantitative macrophages 4 (c); V 3 Qualitative 4	Quantitative hepatocytes 2 (c) Bile ducts 1; V 2 Qualitative 3	Quantitative glomeruli 3 tubules 2; V 3 Qualitative 2
5	Quantitative 0; V 2 Qualitative 1	Quantitative 0; V 1 Qualitative 1	Quantitative macrophages 1 (c); V 2 Qualitative 1	Quantitative 0; V 0 Qualitative 0	Quantitative glomeruli 1 tubules 0; V 1 Qualitative 1
6	Quantitative 0; V 1 Qualitative 2	Quantitative 0; V 2 Qualitative 1	Quantitative macrophages 3 (c); V 2 Qualitative 3	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative Glomeruli 2 tubules 2; V 2 Qualitative 2
7	Quantitative 0; V 3 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative macrophages 3 (c); V 3 Qualitative 3	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative glomeruli 2 tubules 2; V 2 Qualitative 2
8	Quantitative 0; V 3 Qualitative 3	Quantitative 0; V 2 Qualitative 2	Quantitative macrophages 3 (c); V 3 Qualitative 3	Quantitative hepatocytes 2 (c) Bile ducts 2; V 2 Qualitative 2	Quantitative glomeruli 2 tubules 2; V 3 Qualitative 2
9	Quantitative 0; V 1 Qualitative 1	Quantitative 0; V 2 Qualitative 2	Quantitative macrophages 3(c); V 2 Qualitative 2	Quantitative hepatocytes 2 (c) Bile ducts 2; V 1 Qualitative 2	Quantitative glomeruli 2 tubules 1; V 2 Qualitative 1
10	Quantitative 0; V 1 Qualitative 1	Quantitative 0; V 1 Qualitative 1	Quantitative macrophages 1(c); V 2 Qualitative 2	Quantitative 0; V 0 Qualitative 0	Quantitative glomeruli 1 tubules 0; V 1 Qualitative 1
11	Quantitative 0; V 3 Qualitative 3	Quantitative 0; V 3 Qualitative 4	Quantitative macrophages 4 (c); V 3 Qualitative 4	Quantitative hepatocytes 2 (c) Bile ducts 1; V 2 Qualitative 3	Quantitative glomeruli 3 tubules 2; V 3 Qualitative 2
12	Quantitative 0; V 2 Qualitative 1	Quantitative 0; V 1 Qualitative 1	Quantitative macrophages 1 (c); V 2 Qualitative 1	Quantitative 0; V 0 Qualitative 0	Quantitative glomeruli 1 tubules 0; V 1 Qualitative 1
13	Quantitative 0; V 1 Qualitative 2	Quantitative 0; V 2 Qualitative 1	Quantitative macrophages 3 (c); V 2 Qualitative 3	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative glomeruli 2 tubules 2; V 2 Qualitative 2
14	Quantitative 0; V 3 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative macrophages 3 (c); V 3 Qualitative 3	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative gomeruli 2 tubules 2; V 2 Qualitative 2

V, intravascular staining; n, nuclear staining; c, cytoplasm staining. For quantitative analysis, the TREM-1 stained cells were counted and divided by the total number of cells and multiplied x 100. Immunohistochemical positivity score was defined as: 0, nuclear and/or cytoplasm staining absent; 1, nuclear and/or cytoplasmic staining, 25%; 2, nuclear and/or cytoplasmic staining, 50%; 3, nuclear and/or cytoplasmic staining, 75%; 4, nuclear and/or cytoplasm staining, 100%. Immunohistochemical staining in blood vessel was quantified as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable, 100%. Five degrees of staining intensity were defined as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable.

the statistical analysis; a 2-tailed p value ≤ 0.05 was considered statistically significant). Rivera-Chavez and Minei (2009) reported an s-TREM-1 cut-off value of 230pg/ml, which yielded a sensitivity of 98% and specificity of 91% for differentiating patients with SIRS from those with infection. Patients with infection had significantly higher s-TREM-1 concentrations than patients with SIRS (median 398 pg/ml vs. 78 pg/ml, respectively).

Wang and Chen (2011) reported findings concerning 32 septic patients and 24 patients affected by SIRS in intensive care units. Compared to the SIRS group, s-TREM-1 was significantly increased in the sepsis group. In addition to its use as a diagnostic biomarker, s-TREM-1 concentration can used to determine the prognosis of septic patients. Gibot et al. (2005) measured plasma s-TREM-1 concentration together with monocyte expression of TREM-1 in 63 septic patients to determine prognosis.

Promising results with s-TREM-1 as diagnostic sepsis marker have been reported. Brenner et al. (2016) reported that the plasma level of s-TREM-1 was a better biomarker for identifying patients with septic shock than the most commonly used acute phase proteins, CRP and PCT.

Gibot et al. (2007) reported that measurement of serum PCT together with s-TREM-1 concentrations could be useful for detecting nosocomial sepsis. Aksaray et al. (2016) also reported that s-TREM-1 and PCT are excellent markers for early diagnosis of sepsis. Although further studies are required to confirm them, our preliminary findings indicate that s-TREM-1 could enable postmortem diagnosis of sepsis with good sensitivity and specificity.

Table 3. Qualitative and quantitative results for immunohistochemical staining for TREM 1: group 2.

CASE	Brain	Heart	Lung	Liver	Kidney
Case 1	Quantitative 0; V 2 Qualitative 0	Quantitative 0; V 0 Qualitative 0	Quantitative macrophages 1(c); V 2 Qualitative 1	Quantitative 0; V 0 Qualitative 0	Quantitative Glomeruli 0 tubules 1; V 0 Qualitative 1
Case 2	Quantitative 0; V 2 Qualitative 2	Quantitative 0; V 2 Qualitative 2	Quantitative macrophages 2(c); V 2 Qualitative 2	Quantitative hepatocytes 1 (c) Bile ducts 1; V 1 Qualitative 1	Quantitative Glomeruli 2 tubules 2; V 2 Qualitative 1
Case 3	Quantitative 0; V 1 Qualitative 0	Quantitative 0; V 1 Qualitative 1	Quantitative 0 (c); V 1 Qualitative 1	Quantitative 0; V 0 Qualitative 0	Quantitative Glomeruli 0 tubules 1; V 0 Qualitative 1
Case 4	Quantitative 0; V 1 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative 0 (c); V 2 Qualitative 1	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative Glomeruli 2 tubules 0; V 1 Qualitative 2
Case 5	Quantitative 0; V 4 Qualitative 3	Quantitative 0; V 2 Qualitative 3	Quantitative macrophages 3 (c); V 3 Qualitative 2	Quantitative hepatocytes 2 (c) Bile ducts 1; V 2 Qualitative 2	Quantitative Glomeruli 2 tubules 3; V 3 Qualitative 2
Case 6	Quantitative 0; V 2 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative macrophages 2 (c); V 2 Qualitative 2	Quantitative hepatocytes 0 (c) Bile ducts 1; V 0 Qualitative 1	Quantitative Glomeruli 1 tubules 0; V 1 Qualitative 1
Case 7	Quantitative 0; V 3 Qualitative 3	Quantitative 0; V 3 Qualitative 2	Quantitative macrophages 4 (c); V 4 Qualitative 3	Quantitative hepatocytes 3 (c) Bile ducts 2; V 2 Qualitative 3	Quantitative Glomeruli 4 tubules 3; V 2 Qualitative 3
Case 8	Quantitative 0; V 1 Qualitative 2	Quantitative 0; V 2 Qualitative 1	Quantitative macrophages 1 (c); V 2 Qualitative 2	Quantitative hepatocytes 1 (c) Bile ducts 0; V 0 Qualitative 1	Quantitative Glomeruli 2 tubules 2; V 2 Qualitative 1
Case 9	Quantitative 0; V 2 Qualitative 2	Quantitative 0; V 2 Qualitative 2	Quantitative macrophages 2(c); V 2 Qualitative 2	Quantitative hepatocytes 1 (c) Bile ducts 1; V 1 Qualitative 1	Quantitative Glomeruli 2 tubules 2; V 2 Qualitative 1
Case 10	Quantitative 0; V 1 Qualitative 0	Quantitative 0; V 1 Qualitative 1	Quantitative 0 (c); V 1 Qualitative 1	Quantitative 0; V 0 Qualitative 0	Quantitative Glomeruli 0 tubules 1; V 0 Qualitative 1
Case 11	Quantitative 0; V 1 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative 0 (c); V 2 Qualitative 1	Quantitative hepatocytes 1 (c) Bile ducts 0; V 1 Qualitative 2	Quantitative Glomeruli 2 tubules 0; V 1 Qualitative 2
Case 12	Quantitative 0; V 4 Qualitative 3	Quantitative 0; V 2 Qualitative 3	Quantitative macrophages 3 (c); V 3 Qualitative 2	Quantitative hepatocytes 2 (c) Bile ducts 1; V 2 Qualitative 2	Quantitative Glomeruli 2 tubules 3; V 3 Qualitative 2
Case 13	Quantitative 0; V 2 Qualitative 2	Quantitative 0; V 1 Qualitative 2	Quantitative macrophages 2 (c); V 2 Qualitative 2	Quantitative hepatocytes 0 (c) Bile ducts 1; V 0 Qualitative 1	Quantitative Glomeruli 1 tubules 0; V 1 Qualitative 1
Case 14	Quantitative 0; V 3 Qualitative 3	Quantitative 0; V 3 Qualitative 2	Quantitative macrophages 4 (c); V 4 Qualitative 3	Quantitative hepatocytes 3 (c) Bile ducts 2; V 2 Qualitative 3	Quantitative Glomeruli 4 tubules 3; V 2 Qualitative 3

V, intravascular staining; n, nuclear staining; c, cytoplasmic staining. For quantitative analysis, the TREM-1 stained cells were counted and divided by the total number of cells and multiplied x 100. Immunohistochemical positivity score was defined as: 0, nuclear and/or cytoplasm staining absent; 1, nuclear and/or cytoplasmic staining, 25%; 2, nuclear and/or cytoplasmic staining, 50%; 3, nuclear and/or cytoplasmic staining, 75%; 4, nuclear and/or cytoplasm staining, 100%. Immunohistochemical staining in blood vessel was quantified as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable, 100%. Five degrees of staining intensity were defined as: 0, absent; 1, low, 25%; 2, sufficient, 50%; 3, good, 75%; 4, considerable.

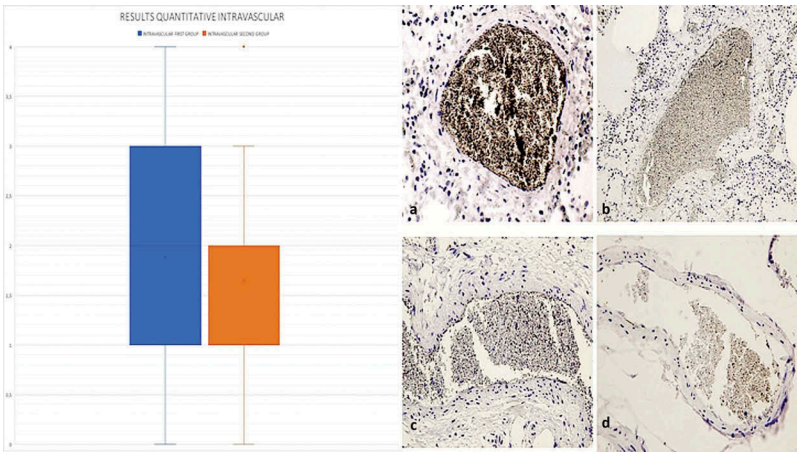

Figure 1. Box and whiskers plot of intravascular immunostaining by anti-TREM-1. Staining of the TREM-1 antibody in blood vessels of kidney a) x 200, lung b) x 100, myocardium c) x 200, and brain d) x 200.

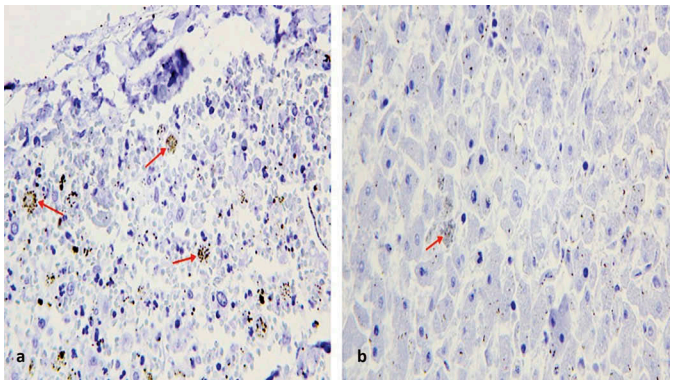

Figure 2. a) TREM-1 staining (arrows) in cytoplasm of the myelomonocyte line. x 60. b) Weak TREM-1 staining (arrow) in hepatocytes. x 200.

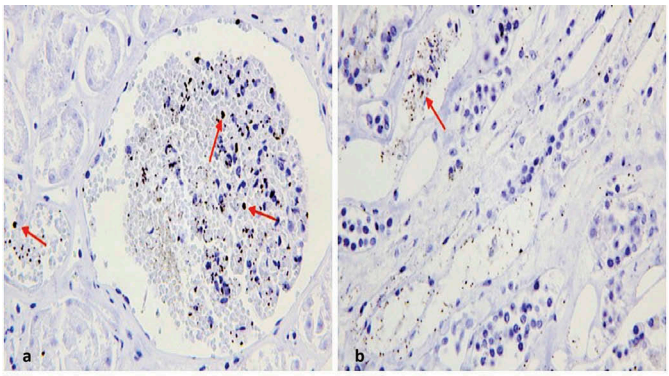

Figure 3. TREM-1 staining (arrows) in glomeruli a) x 100 and renal tubules (arrows) b) x 200.

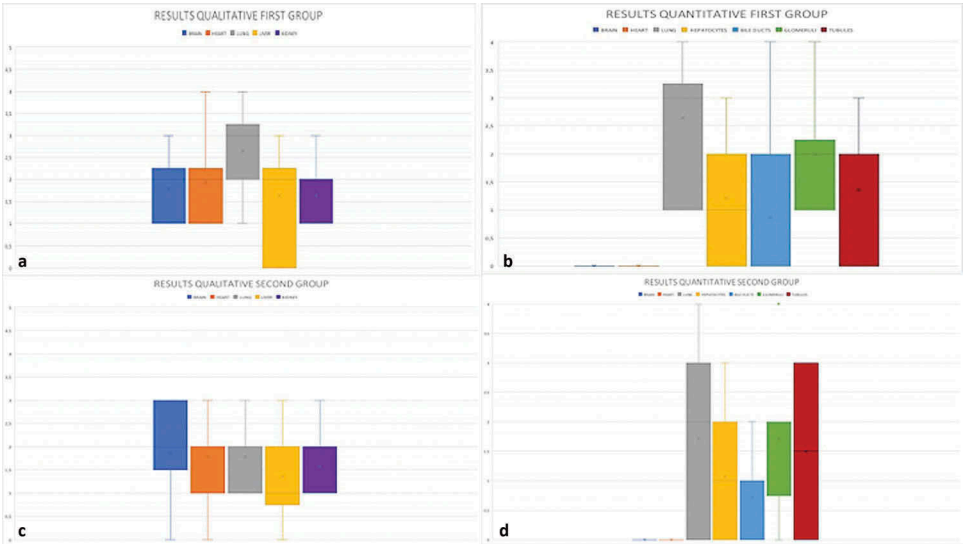

Figure 4. Box and whiskers plot showing greater immunohistochemical reaction, both quantitatively and qualitatively, in the group with documented ante-mortem s-TREM-1.

Our findings for lung samples suggest that s-TREM-1 might be produced principally in the cells of the monocyte line. Our findings for liver and kidney samples could be related to the pathway of excretion of TREM-1. TREM-1 staining in kidney tubules, hepatocytes and liver ducts are a normal physiological finding related to the elimination of this protein from the circulation.

One limitations of our study is our use of a relatively small number of subjects. Precise criteria, however, were used for selecting subjects. Also, detailed immunohistochemical studies TREM-1 are not available, which precluded comparison of our observations with others.

We believe that our measurement of s-TREM-1 may be promising for making postmortem diagnoses of sepsis. Our immunohistochemical technique currently is more convenient compared to searching for s-TREM-1 in serum or other biological fluids (Bellos et al. 2018). It is important to compare the diagnostic accuracy of s-TREM-1 with other biomarkers, such as CRP and PCT. the variation in the threshold values used (between 77.5 and 1707.35 pg/ml), however, limit the value of these findings in clinical practice (Bellos et al. 2018). Also TREM-1 could be stained in patients with non-infective inflammatory diseases such as psoriasis, ulcerative colitis or immune complex vasculitis. Moreover, TREM-1 could be stained in patients affected by hemorrhagic shock, reperfusion injury or by a peptic ulcer caused by *H. pylori* (Gibot et al. 2008; Yasuda et al. 2008; Barbatzas and Pimentel 2011).

If s-TREM-1 staining can be found in cases un related to sepsis, the anti-TREM-1 antibody could be combined with an anti-procalcitonin and/or lactoferrin antibody to produce considerable sensitivity and specificity in sepsis related cases of death (Maiese et al. 2017; Galassi et al. 2018). Immunohistochemical staining using the anti-TREM-1 antibody can be a useful method for postmortem diagnosis of sepsis (Palmiere et al. 2013).

Disclosure statement

No potential conflict of interest was reported by the authors.

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