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Letter to the Editor

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Measurement of apolipoprotein M in sepsis-related deaths

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To the Editor,

Systemic inflammation and generalized infection have been shown to be accompanied by significant cytokine-mediated alterations in lipid and lipoprotein metabolism [1, 2]. Apolipoprotein M (ApoM) is a member of the lipocalin protein family. Under normal conditions, most of the ApoM in plasma is associated with high-density lipoprotein (HDL). In humans, plasma ApoM concentration is approximately 0.9 μmol/L (23 mg/L) and correlates positively with plasma HDL, low-density lipoprotein (LDL), and total cholesterol [1, 3–5].

Previous studies indicated that ApoM concentrations are decreased in patients with sepsis, thus suggesting that ApoM may behave as a negative acute-phase reactant [1, 3]. In the study herein described, ApoM levels were measured in postmortem serum from femoral blood in a series of sepsis-related fatalities and control cases that were subjected to medicolegal investigations. Our aim was to determine whether ApoM could be determined in postmortem serum collected during autopsy. We furthermore wished to assess its diagnostic potential in identifying sepsis-related deaths.

Two study groups were prospectively formed, a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of 20 cases. All individuals included in this group had been admitted to the intensive care unit of the local hospitals where they died. All cases had a documented, clinical diagnosis of sepsis in vivo (duration between 18 and 96 h). Sepsis was diagnosed on the basis of evidence of infection along with the presence of systemic inflammatory response syndrome, according to the definition of the American College of Chest Physicians/Society of Critical Care Medicine [6].

Autopsies, histology, toxicology, biochemistry, and microbiology were performed in all cases. Postmortem intervals ranged between 6 and 62 h. ApoM measurement was systematically carried out in postmortem serum obtained from femoral blood. Sepsis and multiple organ dysfunction syndrome as causes of death were confirmed by postmortem investigations in all cases. Alternative causes of death were excluded on the basis of postmortem investigation results. According to medical records as well as antemortem and postmortem biochemical results, none of these subjects had diabetes mellitus or lipid disorders. The descriptive characteristics of the studied subjects are reported in Table 1.

The control group consisted of 20 age-, race-, and sex-matched forensic autopsy cases. None of the subjects included in this group had a documented, clinical diagnosis of sepsis in vivo and none had been admitted to the hospital before death. Autopsies, histology, toxicology, biochemistry, and microbiology were performed between 8 and 64 h after death. Postmortem investigations failed to reveal findings consistent with the existence of underlying bacterial infections. According to medical records and postmortem biochemical results, none of these subjects had diabetes mellitus or lipid disorders. Complications of diabetes mellitus as causes of death were also excluded. The descriptive characteristics of the studied subjects are reported in Table 1.

Personal data pertaining to both studied groups were collected from clinical patient databases and medical records obtained from general practitioners and local health services. In all sepsis cases, antemortem clinical data were only collected after having performed

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Table 1 Descriptive characteristics of the studied subjects.

Sepsis cases	Sex and age, years	Body weight, kg	Height, cm	BMI, kg/m²	Control cases	Sex and age, years	Body weight, kg	Height, cm	BMI, kg/m²
1	Male 48	75	178	23.7	1	Male 49	74	177	23.9
2	Male 54	80	181	24.4	2	Male 55	81	182	24.5
3	Male 39	74	176	23.9	3	Male 40	75	178	23.7
4	Female 62	62	168	22.0	4	Female 62	60	169	21.0
5	Female 68	59	166	21.4	5	Female 67	60	170	20.8
6	Male 49	77	177	24.6	6	Male 48	76	175	24.8
7	Male 50	78	180	24.1	7	Male 51	75	179	23.4
8	Female 51	52	159	20.6	8	Female 50	51	158	20.4
9	Male 50	80	185	23.4	9	Male 49	81	187	23.2
10	Male 67	81	182	24.5	10	Male 66	78	178	24.6
11	Male 69	75	175	24.5	11	Male 69	74	176	23.9
12	Female 44	59	160	23.0	12	Female 45	56	159	22.2
13	Female 65	54	158	21.6	13	Female 64	55	156	22.6
14	Male 60	73	174	24.1	14	Male 61	74	178	23.4
15	Male 59	79	182	23.8	15	Male 60	77	177	24.6
16	Female 71	59	170	20.4	16	Female 72	60	172	20.3
17	Male 70	84	184	24.8	17	Male 71	78	185	22.8
18	Male 49	78	179	24.3	18	Male 48	74	175	24.2
19	Male 46	81	183	24.2	19	Male 46	77	181	23.5
20	Female 51	56	161	21.6	20	Female 51	57	162	21.7

postmortem investigations. Procalcitonin (PCT) and C-reactive protein (CRP) were measured in all sepsis cases during hospitalization.

As all cases selected for the control group originated from forensic practice with deaths occurring outside the hospital in all cases, laboratory analysis results shortly before death were unavailable. PCT, CRP, lipopolysaccharide-binding protein (LBP), ApoM, HDL-cholesterol, LDL-cholesterol, and total cholesterol were systematically measured in postmortem serum from femoral blood.

Peripheral blood was collected by aspiration through the femoral vein(s) during autopsy. Blood was centrifuged immediately after collection at 3000 *g* for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. PCT, CRP, and LBP concentrations were determined according to the techniques previously described [7, 8]. The stability of PCT, CRP, and LBP in postmortem serum was assumed on the basis of information from currently available forensic literature [7, 8]. ApoM was measured in postmortem serum with a commercialized specific enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer protocol (Human Apolipoprotein M, APOM ELISA kit; CUSABIO, Wuhan, China). Results were expressed in µmol/L and mg/L. ApoM cutoff values in postmortem serum were not preliminarily identified owing to the unavailability of previous studies on postmortem material for comparison. HDL-cholesterol, LDL-cholesterol, and total cholesterol were measured in postmortem serum from femoral blood using an enzymatic colorimetric method (CHOD-PAP method). Results were expressed in mmol/L and mg/dL.

Non-parametric tests were used throughout the study. The Mann-Whitney U-test was used to evaluate the differences between sepsis and control cases. A p-value of <0.05 was considered significant for all tests. Graphpad Prism 4.0 (Graphpad Software, La Jolla, CA, USA) was used for statistics.

Table 2 summarizes the ranges, mean values, medians, and standard deviations (SDs) for all tested parameters in both studied groups. As expected, postmortem serum PCT, CRP, and LBP values were significantly higher in the sepsis group ($p<0.0001$) and were consistent with the presence of generalized inflammation and bacterial infection. Total cholesterol, LDL-cholesterol, and HDL-cholesterol behaved comparably in both studied groups, with significantly lower levels in septic cases ($p<0.05$). ApoM values in the septic group were significantly lower ($p<0.0001$). No correlation between ApoM values and postmortem intervals was observed in either sepsis or control cases. Lastly, postmortem serum PCT and CRP concentrations were systematically lower than the corresponding antemortem values, as expected on the basis of the available forensic literature.

Table 2 Ranges, mean values, medians, and SD for all tested parameters in both studied groups.

	PCT, µg/L	CRP, mg/L	LBP, µg/mL	HDL, mmol/L (mg/dL)	LDL, mmol/L (mg/dL)	Total cholesterol, mmol/L (mg/dL)	ApoM, µmol/L (mg/dL)
Septic group (n=20)							
Ranges	1.99–6.16	65–235	11–50	0.39–1.17 (15–45)	1.42–2.46 (55–95)	2.46–3.39 (95–131)	0.23–0.74 (6–19)
Mean	3.20	94.50	20.50	0.79 (30.50)	1.97 (76.00)	2.83 (109.50)	0.47 (12.00)
Median	3.37	108.00	24.10	0.78 (30.00)	1.95 (75.25)	2.85 (110.20)	0.47 (12.10)
SD	1.12	40.27	10.60	0.20 (7.71)	0.24 (9.11)	0.24 (9.27)	0.12 (3.13)
Controls (n=20)							
Ranges	0.06–0.19	3–12	3–12	0.47–3.11 (18–120)	2.59–3.89 (100–150)	4.09–5.70 (158–220)	0.78–1.25 (20–32)
Mean	0.07	8.50	5.00	1.20 (46.50)	3.25 (125.50)	4.92 (190.00)	1.04 (26.50)
Median	0.08	7.85	5.05	1.32 (50.95)	3.25 (125.60)	4.89 (188.80)	1.04 (26.45)
SD	0.04	2.13	2.06	0.67 (25.75)	0.33 (12.57)	0.54 (20.84)	0.14 (3.56)

Abbreviations are reported in the text.

Cytokines produced during systemic inflammation are known to mediate significant modifications in lipid and lipoprotein metabolism. Triglyceride and very low density lipoprotein (VLDL) increases are commonly observed during inflammation and are the consequences of both decreased triglyceride clearance and enhanced VLDL production. Furthermore, total cholesterol, LDL, and HDL decreases are also commonly found. Inflammatory HDL particles are increased in size and modified in lipid profile, metabolism, and function. As a result of these changes, HDL loses its antioxidant and anti-inflammatory properties [1, 3, 9].

ApoM was speculated as contributing to the anti-inflammatory properties of HDL as soon as it was identified as one of its components [3–5, 9]. Indeed, inhibition of ApoM production was demonstrated to result in decreased HDL levels, increased HDL size, and impaired transport of cholesterol out of macrophages to HDL. Conversely, ApoM overexpression was demonstrated to limit atherosclerotic lesion size [1, 3, 5, 9]. The results of our analyses seem to indicate that, as in the clinical field, decreased ApoM concentrations can be measured in septic cases investigated after death. Analogously, decreases in total cholesterol, HDL-cholesterol, and LDL-cholesterol that may characterize severe sepsis and septic shock in the living can also be identified in postmortem samples.

Although our research has yielded some interesting findings, the limitations of our study must be acknowledged. The first is the relatively small number of subjects, which may limit research accuracy. A further limit concerns the present unavailability of detailed studies on ApoM behavior in the postmortem setting, rendering our observations mostly speculative.

To conclude, the study herein presented is the first attempt at measuring ApoM values in postmortem serum in septic and control cases that were subjected to medicolegal investigations. Within the aforementioned limits, the results of our investigations indicate that ApoM can be measured in postmortem samples and may be a useful biomarker in identifying sepsis-related deaths.

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