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Elevated plasma CL-K1 level is associated with a risk of developing disseminated intravascular coagulation (DIC)

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Abstract

Collectin kidney 1 (CL-K1) is a recently identified collectin that is synthesized in most organs and circulates in blood. CL-K1 is an innate immune molecule that may play a significant role in host defense. As some collectins also play a role in coagulation, we hypothesized that an effect of CL-K1 may be apparent in disseminated intravascular coagulation (DIC), a gross derangement of the coagulation system that occurs in the setting of profound activation of the innate immune system. DIC is a grave medical condition with a high incidence of multiple organ failure and high mortality and yet there are no reliable biomarkers or risk factors. In our present study, we measured plasma CL-K1 concentration in a total of 659 specimens, including 549 DIC patients, 82 non-DIC patients and 27 healthy volunteers. The median plasma CL-K1 levels in these cohorts

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were 424, 238 and 245 ng/ml, respectively, with no significant difference in the latter two groups. The incidence of elevated plasma CL-K1 was significantly higher in the DIC patients compared to the non-DIC patients, resulting in an odds ratio of 1.929 (confidence interval 1.041–3.866). Infection, renal diseases, respiratory diseases, and cardiac diseases were more frequently observed in the DIC group than in the non-DIC group. In the DIC group, vascular diseases were associated with elevated plasma CL-K1 levels while age and acute illness had little effect on plasma CL-K1 levels. Independent of DIC, elevated plasma CL-K1 levels were associated with respiratory disease and coagulation disorders. These results suggest that specific diseases may affect CL-K1 synthesis in an organ dependent manner and that elevated plasma CL-K1 levels are associated with the presence of DIC. Further investigations in cohorts of patients are warranted. We propose that elevated plasma CL-K1 may be a new useful risk factor and possibly biomarker for the prediction of developing DIC.

Keywords

Innate immunity; Collectin; Collectin kidney 1 (CL-K1); Disseminated intravascular coagulation; Respiratory diseases; Coagulation disorders; Vascular diseases

Introduction

The body’s first line of host defense is the innate immune system, which includes complement proteins, coagulation enzymes and pattern recognition molecules [1], as well as effectors cells such as macrophages. Collectin kidney 1 (CL-K1), also known as collectin 11, is a pattern recognition molecule of the complement system, and is a member of the collectin family, which also includes mannose binding lectin (MBL) and lung surfactant proteins [2, 3]. These latter collectins were identified in the late 1980s and have been extensively investigated in both laboratory and clinical studies [4, 5]. Little is known about CL-K1, which was first identified in 2006 [2], and reliable antibodies required for analysis have only recently become available [6, 7]. Average plasma CL-K1 concentrations have been reported as 340 and 284 ng/ml among healthy Japanese and Danish populations, respectively [6, 7], which are approximately comparable. This is much lower than the reported MBL plasma concentration of 2 μg/ml [8, 9], although the effects of this difference in plasma concentration may be diminished by differences in their synthesis, as MBL is produced primarily in the liver whereas CL-K1 is synthesized in a much wider distribution of tissues, including liver, lung, kidney, brain, and endothelial cells [2, 10, 11].

CL-K1, like other collectins, recognizes and binds to specific chemical components of microbes [2, 12]. Based on these observations, it has been speculated that CL-K1 may share similar biologic functions with other collectins, in particular MBL. Our earlier investigations have shown that MBL-associated serine protease (MASP)-1/3 in a complex with MBL activates coagulation through a thrombin-like serine protease activity [13–16]. Like MBL, CL-K1 has also been found in a complex with MASP-1/3, supporting our idea that CL-K1 also may be involved with coagulation [2, 12].

Disseminated intravascular coagulation (DIC) is a severe medical complication in which thrombosis (coagulation) and bleeding occur simultaneously. There is a strong association

with multiple organ failure and a very high mortality rate [17–19]. An early stage of DIC can be revealed by a sensitive and specific test that identifies an abnormal biphasic waveform in activated partial thromboplastin time (aPTT) testing [17, 18]. The waveform represents the change in light transmittance through a plasma specimen as the aPTT reaction takes place. The biphasic waveform is characterized by an initial steep negative slope, which is not seen in a normal waveform and is caused, in part, by precipitation of a C-reactive protein (CRP) complex [17, 18, 20]. The abnormal biphasic waveform was predictive of approximately half of fully developed DIC 18 h prior to the diagnosis provided by a battery of several other clinical tests that are currently regarded as definitive of DIC [21]. The waveform is a good test for DIC, but it is well-described and available only on the MDA analyzer which is not routinely available to most laboratories. Therefore, discovering other markers for DIC would be beneficial. Not all patients with similar clinical conditions develop DIC, suggesting that genetic components are involved. While infection and trauma have been associated with presence of the abnormal biphasic waveform, no risk factor or biomarker specific to developing DIC has been identified [17, 18].

Based on these observations, we hypothesized that plasma CL-K1 levels might have an effect in the development of DIC and we examined the relationship between plasma CL-K1 levels and the presence of DIC. Our findings are described below, and we discuss their clinical implications.

Materials and methods

Plasma samples and patient diagnoses

Citrated plasma samples were collected and stored at −80 °C; a total of 659 specimens included 549 patients with DIC, 83 patients without DIC and 27 healthy volunteers. Although samples were collected prospectively, the analysis was retrospective, using clinical data retrieved from the hospital electronic medical records and surplus plasma samples from the clinical laboratories.

DIC was diagnosed by the presence of an abnormal biphasic waveform from aPTT testing, as described above [18]. The waveform is 98 % specific for DIC [22], unlike other laboratory tests which lack specificity for DIC. A biphasic waveform is characterized by a steeply negative initial slope, called slope_1 (%T/s). Abnormal slope_1 is defined as less than −0.1 %T/s, with a normal slope_1 defined as between −0.1 and +0.1, with reference values determined in the Coagulation Laboratory at the Massachusetts General Hospital. The aPTT waveform analysis was performed on an MDA-II coagulation analyzer (Tcoag/Diagnostica Stago, Parsippany NJ). The MDA Coagulation Analyzer uses waveform analysis technology to automatically detect the presence of this abnormal waveform while running routine aPTT tests using Platelin L reagent [18].

Non-DIC patients were randomly selected hospital patients who did not have a clinical diagnosis of DIC, all of whom also had a normal aPTT waveform. In determining the DIC and non-DIC patients' medical conditions, respiratory diseases included acute respiratory distress syndrome (ARDS), other respiratory distress, respiratory failure, and pulmonary emboli. Vascular diseases included cerebrovascular disease and peripheral vascular diseases

exclusive of coronary artery diseases, which were separately categorized as cardiac diseases. Coagulation disorders included any disorder of hemostasis including retroperitoneal, gastrointestinal, cerebrovascular or other hemorrhage, pulmonary emboli, deep vein thrombosis, heparin-induced thrombocytopenia, thrombosis, or stroke (ischemic, embolic or hemorrhagic). Gastrointestinal and renal diseases were also recorded, as well as diabetes and infection.

ELISA assays

Plasma CL-K1 was assayed using a previously established sandwich ELISA method using two CL-K1 specific antibodies, with minor modifications [6]. Briefly, 384 well plates were coated with 20 μl of an anti-CL-K1 rabbit polyclonal antibody. After rinsing and blocking, 20 μl of diluted plasma samples and standard were incubated in duplicate. All plasma samples used in this study were thawed only once. After rinsing, the wells were incubated with a biotinylated anti-CL-K1 monoclonal antibody followed by ABC-AP (Vector Lab, Burlingame, CA) and then developed using p-nitrophenyl phosphate substrate (Sigma-Aldrich). Reactions were assayed for absorbance at 405 nm using an M3 plate reader (Molecular Devices).

From assays performed using plasma specimens from 27 healthy volunteers, the median of plasma CL-K1 levels was 245 ng/ml with interquartile range 160–273 ng/ml. The highest concentration measured in this group was 772 ng/ml. The CL-K1 reference range was found to be <619 ng/ml (normal donor mean + 2SD).

Plasma samples from a subpopulation of 216 patients (201 DIC and 15 non-DIC) were also assayed for plasma CRP, an acute phase protein, using ELISA (DuoSet, R&D Systems), according to the manufacturer's instructions.

Statistics

Statistical analysis was performed using JMP software (SAS Institute, Cary, NC), and specific methods used are indicated in the table and figures. For a logistic regression analysis, plasma CL-K1 concentration was assigned to low and high, using a cut off of 619 ng/ml (mean + 2SD of healthy normal volunteers [23, 24]). The cut-off of 619 ng/mL was determined from our normal range study described above. As specified in the text below, p values less than 0.05 were considered to be statistically significant.

Results

Analysis of diseases associated with the presence of DIC

The median age and gender ratios were similar between the DIC and the non-DIC patient groups (Table 1). These data do not reveal an association between age and gender and the presence of DIC. Compared to the non-DIC group, the DIC group patients had a significantly higher incidence of several diseases. Ranked from the highest incidence in our sample to the lowest, these included: infection, renal diseases, respiratory diseases, and cardiac diseases (Table 1).

Plasma CL-K1 levels among the non-DIC patients are similar to normal controls

Plasma CL-K1 levels in the non-DIC group had a median of 176 ng/ml (Table 2), which were not statistically different from the healthy normal group which had a median of 245 ng/ml. The highest plasma CL-K1 levels sampled were also comparable, as these were measured at 772 and 869 ng/ml in the healthy normal and the non-DIC groups, respectively. These findings suggest that the non-DIC group was similar to normal controls with respect to plasma CL-K1 levels, and that the non-DIC patients could be used as a control group.

Elevated plasma CL-K1 level is associated with the presence of DIC

In contrast, plasma CL-K1 levels in the DIC group were significantly higher than the non-DIC group (Fig. 1a). A similar trend was observed when plasma CL-K1 levels were compared in different gender groups with statistical significance (Fig. 1b). In contrast, when plasma CL-K1 levels were compared for gender difference in the DIC or the non-DIC group (male DIC vs. female DIC or male non-DIC vs. female non-DIC), there was no statistical difference ($p = 0.655$ and 0.856, respectively) (Fig. 1b). The median CL-K1 levels were more than 2 times higher in the DIC groups than the non-DIC groups, regardless of gender (Table 2).

We further analysed characteristics of the elevated plasma CL-K1 levels within the DIC and the non-DIC groups. A threshold for elevated plasma CL-K1 level was established as greater than 619 ng/ml, and logistic nominal regression analysis was used to evaluate the statistical significance of the high CL-K1 level. Plasma CL-K1 levels were significantly higher in the DIC group than the non-DIC group in all subjects, and there was a statistically significant excess of subjects with high CL-K1 levels (Table 2). A similar association was also observed in the male group, although this was not statistically significant in the female group (Table 2). Accordingly, the odds ratios (OR) were statistically significant for high CL-K1 plasma levels in all DIC subjects considered together and among male subjects, although the OR for high CL-K1 among DIC patients was not statistically significant among female subjects (Table 2).

In the DIC group, the effect of age on plasma CL-K1 levels was assessed by cohorts defined by grouping subjects into 20-year age intervals from 21 to 100 years old. Patients younger than 20 years of age were excluded due to small sample size, as there were only 17 subjects in that cohort and consequently statistical significance could not be achieved. As shown in Table 3, the median plasma CL-K1 concentrations were between 369 and 443 ng/ml and no statistical difference was obtained among different age cohorts. Likewise, the incidence of elevated plasma CL-K1 levels was between 21.5 and 34.3 %, again with no statistically significant difference among the 4 age groups. These data demonstrate that in the DIC group, plasma CL-K1 levels and the incidence of elevated plasma CL-K1 concentrations were similar across age groups ranging from 21 to 100 years old.

Effect of clinical factors on plasma CL-K1 levels

We also analysed the association of other diseases and plasma CL-K1 levels, in patients without or with DIC, by comparing the incidence of elevated plasma CL-K1 levels between the non-DIC and the DIC groups with respect to the most commonly identified co-existing

diseases (Table 4). A statistically significant higher incidence of elevated plasma CL-K1 levels was observed only in patients with vascular diseases. Among all patients, both those without and with DIC, there was a statistically significant increased incidence of high plasma CL-K1 levels in patients with respiratory diseases and coagulopathies (Table 5).

The effects of acute illness were also analyzed for a subpopulation of patients. Acute illness was defined as plasma CRP levels more than 8 μg/ml (a reference value established by the clinical laboratory at Massachusetts General Hospital). The median CRP for the non-DIC and the DIC group was 0.7 (interquartile range 0–21.3) μg/ml and 36.5 (interquartile range 2.2–102) μg/ml, respectively. In patients with DIC, the incidence of elevated plasma CRP levels was 138 out of 201 (68.7 %), which was significantly higher than the 5 out of 15 (33.3 %) patients with elevated CRP in a group of patients without DIC (p= 0.0090, Fisher's Exact Test). In comparison, the incidence of elevated plasma CL-K1 levels in patients with high versus normal CRP levels was 55/138 (40.0 %) and 24/63 (28.1 %), respectively, which is not statistically significant (p = 0.88, Fisher's Exact Test). These data suggest that although acute illness, defined by elevated plasma CRP levels, is associated with a higher incidence of DIC, acute illness is not associated with higher plasma CLK1 levels.

Discussion

CL-K1 was initially discovered in 2006, and there is a relative dearth of information regarding its biological function or clinical relevance [2]. The recent availability of anti-CL-K1 antibodies, which are required to perform ELISA and other immunochemical analyses [6, 7], provide an important foundation for advancing both basic scientific and clinical investigation of CL-K1. In this current study, we measured the plasma CL-K1 concentration among healthy Americans, and found it to be 265 ± 177 ng/ml, which is in the range of prior measurements of 340 ± 130 ng/ml measured in healthy Japanese and 284 ng/ml measured in healthy Danes [6, 7]. This result establishes that plasma CL-K1 concentrations in healthy Americans are similar to those Japanese and Danes. However, studies in other ethnic groups could demonstrate additional factors that may influence plasma CL-K1 levels.

The current study demonstrates that among non-DIC patients, CL-K1 levels are comparable to a healthy population of normal control subjects. In contrast, there is a higher average plasma CL-K1 level and a higher incidence of elevated plasma CL-K1 in DIC patients. In the DIC group, cardiac, renal, and infectious diseases were frequently observed, however these conditions did not show an association with elevated plasma CL-K1 levels (as shown in tables 1 and 5). Regarding renal diseases, plasma CL-K1 levels among dialysis patients are not elevated (Wakamiya N, unpublished observations), suggesting that renal disease alone does not increase plasma CL-K1 levels. In addition, infection, cancer, diabetes, and vascular or gastrointestinal disorders were not significantly associated with elevated CL-K1 levels in our study. Further study is warranted to examine a potential association with respiratory disorders. Our results also reveal no statistically significant effect of age on plasma CL-K1 levels among patients with DIC, although age has been identified as an important associated factor in the development of other clinical complications [25]. Taken together, these results suggest that elevated plasma CL-K1 levels maybe a biomarker for developing DIC.

DIC is a serious medical condition in which thrombosis (coagulation) and bleeding occur simultaneously. It has a strong association with multiple organ failure and very high mortality [17–19]. Our results show that coagulation disorders are associated with elevated plasma CL-K1 levels independent of the presence of DIC, supporting the idea that CL-K1 may play a role in coagulation. In support of the hypothesis that collectins may play a role in both coagulation and immunity, our other studies have demonstrated that MBL, also a collectin molecule, has a thrombin-like serine protease activity when it forms a complex with MASP-1/3 [13, 15, 16, 26]. This MBL-MASP-1/3 complex also activates the lectin complement pathway [14, 27, 28], demonstrating its role in the immune system. Intriguingly, a complex of CL-K1 and MASP-1/3 has been found in circulation that is capable of complement activation [2, 12]. Taken together, these observations lead to our hypothesis that a complex of CL-K1 and MASP-1/3 may mediate coagulation [2, 12]. Although we present data here that reveal an association between elevated CL-K1 and the coagulopathic DIC condition, further detailed studies are required in order to elucidate the mechanisms by which CL-K1, MASPs or their complexes activate and modulate coagulation.

CL-K1, like other collectins, recognizes and binds to specific chemical components found on microbes [2, 12]. Microbial infection is strongly associated with coagulation disorders. In our earlier work we have demonstrated one manifestation of coagulopathy in DIC, the presence of a biphasic aPTT waveform, which provides an early and sensitive diagnostic test for DIC [17, 18]. In this regard, the results presented here confirm our previous findings, as infection is strongly associated with DIC. Additionally, the results presented here demonstrate a trend toward increased plasma CL-K1 in infection, independent of DIC, although this did not meet a threshold for statistical significance. These observations taken together inform our larger hypothesis that the interactions among CL-K1, MASPs and microbes contribute to abnormal activation of coagulation.

One weakness of this study is that the clinical diagnoses were derived from the medical record, and were established by many different practitioners using a variety of clinical and laboratory data. An additional weakness related to the retrospective methodology is that there is not baseline data for each patient, and changes in CL-K1 could not be assessed longitudinally throughout the clinical course. However, to ensure that accurate results were obtained, aPTT waveforms were measured in a proper and timely manner and all plasma samples were collected contemporaneous with the inpatient admission. Measurement of CL-K1 was performed using ELISAs that are routinely performed in our laboratory, similar to many of our previously reported experiments [13, 29–32]. Therefore, despite the limitations described above, the results shown here are the first to show a relationship between plasma CL-K1 levels and DIC. This association warrants further investigation, and may yield important insights into the connections between immunity and coagulation.

We note that in contrast to the average MBL plasma level of 2 μg/ml, CL-K1 has a significantly lower plasma concentration [6, 8, 9, 33]. However, CL-K1 is synthesized by most organs, including the lung and vascular wall lining endothelial cells [2, 10, 11, 34], in contrast to MBL, which is produced primarily in the liver. Interestingly, a high incidence of elevated plasma CL-K1 levels is associated with respiratory diseases but not with vascular

diseases. However, vascular diseases were commonly seen in association with DIC. We speculate that the ubiquitous presence of CL-K1 throughout the body may contribute to its ability to participate in a systemic response, even though circulating levels of CL-K1 are relatively low. Consequently, and consistent with the observations presented here, CL-K1 may play a key role throughout the host defense and coagulation systems.

It is not known whether CL-K1 is an acute phase protein or how its synthesis is modulated, reflecting the relative paucity of published data. Our study found similar levels of CL-K1 in healthy control subjects and hospital patients without DIC, as well as similar levels of CL-K1 among DIC patients with and without elevated CRP. Since CRP is a sensitive marker for acute phase reactions, our results suggest that CL-K1 is not an acute phase protein. Prospective studies with longitudinal measurements in well-defined patient cohorts would be useful to further establish these findings. Since this is the first study to investigate an association between plasma CL-K1 and DIC, replication of these findings by other researchers is needed before considering the findings to be definitive.

In contrast to the recent report on the genetic deficiency of CL-K1, our results suggest that regulatory gene sequences most likely exist and contribute to increased production and/or secretion of the CL-K1 protein [11]. Further investigations are required to understand the molecular mechanisms involved in this regulation, such as the specific stimuli, mediators and effector cells of increased CL-K1 production.

D-dimer levels were not used in this study because, although it is a sensitive test for DIC, it lacks specificity for DIC. For example, we have shown that 83 % of inpatients undergoing D-dimer testing in our hospital test positive for D-dimers, due to a variety of causes including surgery, thrombosis, and liver dysfunction. Further study is indicated to determine if CL-K1 is a more specific marker for DIC than is D-dimer. The aPTT waveform is sensitive and specific for DIC, although one potential advantage of CL-K1 over the waveform is that the waveform is not routinely available on most coagulation analyzers.

In conclusion, we propose that elevated plasma CL-K1 level may be a useful new biomarker for the prediction of developing DIC. Since this is the first study to investigate an association between plasma CL-K1 and DIC, replication of these findings by other researchers is needed before considering the findings to be definitive. Further investigations are warranted in cohorts of patients in order to understand the mechanisms by which CL-K1 interacts with the coagulation and immune systems. Nevertheless, CL-K1, as a member of the collectin family of the innate immune system, may play an important role in host defense and the maintenance of homeostasis in health and disease.

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References

1. Thiel S, Takahashi K (2013) Collectins In: Encyclopedia of life sciences. Wiley, Chichester, UK
2. Keshi H, Sakamoto T, Kawai T, Ohtani K, Katoh T, Jang SJ, Motomura W, Yoshizaki T, Fukuda M, Koyama S, Fukuzawa J, Fukuoh A, Yoshida I, Suzuki Y, Wakamiya N (2006) Identification and characterization of a novel human collectin CL-K1. Microbiol Immunol 50(12):1001–1013 [PubMed: 17179669]
3. Ohtani K, Suzuki Y, Wakamiya N (2012) Biological functions of the novel collectins CL-L1, CL-K1, and CL-P1. J Biomed Biotechnol 2012:493945. doi:10.1155/2012/493945 [PubMed: 22570530]
4. Thiel S (2007) Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol 44(16):3875–3888 [PubMed: 17768106]
5. Takahashi K (2008) Lessons learned from murine models of mannose-binding lectin deficiency. Biochem Soc Trans 36(Pt 6):1487–1490. doi:10.1042/BST0361487 [PubMed: 19021581]
6. Yoshizaki T, Ohtani K, Motomura W, Jang SJ, Mori K, Kitamoto N, Yoshida I, Suzuki Y, Wakamiya N (2012) Comparison of human blood concentrations of collectin kidney 1 and mannanbinding lectin. J Biochem 151(1):57–64. doi:10.1093/jb/mvr114 [PubMed: 21893516]
7. Selman L, Henriksen ML, Brandt J, Palarasah Y, Waters A, Beales PL, Holmskov U, Jorgensen TJ, Nielsen C, Skjodt K, Hansen S (2012) An enzyme-linked immunosorbent assay (ELISA) for quantification of human collectin 11 (CL-11, CL-K1). J Immunol Methods 375(1–2):182–188. doi:10.1016/j.jim.2011.10.010 [PubMed: 22301270]
8. Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC (2000) Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. J Immunol Methods 241(1–2):33–42 [PubMed: 10915847]
9. Garred P, Larsen F, Madsen HO, Koch C (2003) Mannose-binding lectin deficiency—revisited. Mol Immunol 40(2–4):73–84 [PubMed: 12914814]
10. Uemura K, Saka M, Nakagawa T, Kawasaki N, Thiel S, Jensenius JC, Kawasaki T (2002) L-MBP is expressed in epithelial cells of mouse small intestine. J Immunol 169(12):6945–6950 [PubMed: 12471128]
11. Rooryck C, Diaz-Font A, Osborn DP, Chabchoub E, Hernandez-Hernandez V, Shamseldin H, Kenny J, Waters A, Jenkins D, Kaissi AA, Leal GF, Dallapiccola B, Carnevale F, Bitner-Glindzicz M, Lees M, Hennekam R, Stanier P, Burns AJ, Peeters H, Alkuraya FS, Beales PL (2011) Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nat Genet 43(3):197–203. doi:10.1038/ng.757 [PubMed: 21258343]
12. Hansen S, Selman L, Palaniyar N, Ziegler K, Brandt J, Kliem A, Jonasson M, Skjoedt MO, Nielsen O, Hartshorn K, Jorgensen TJ, Skjodt K, Holmskov U (2010) Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity. J Immunol 185(10):6096–6104. doi:10.4049/jimmunol.1002185 [PubMed: 20956340]
13. Takahashi K, Chang WC, Takahashi M, Pavlov V, Ishida Y, La Bonte L, Shi L, Fujita T, Stahl GL, Van Cott EM (2011) Mannose-binding lectin and its associated proteases (MASPs) mediate coagulation and its deficiency is a risk factor in developing complications from infection, including disseminated intravascular coagulation. Immunobiology 216(1–2):96–102. doi:10.1016/j.imbio.2010.02.005 [PubMed: 20399528]
14. Sekine H, Takahashi M, Iwaki D, Fujita T (2013) The role of MASP-1/3 in complement activation. Adv Exp Med Biol 735:41–53 [PubMed: 23402018]
15. Gulla KC, Gupta K, Krarup A, Gal P, Schwaeble WJ, Sim RB, O'Connor CD, Hajela K (2010) Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. Immunology 129(4):482–495. doi:10.1111/j.1365-2567.2009.03200.x [PubMed: 20002787]
16. Presanis JS, Hajela K, Ambrus G, Gal P, Sim RB (2004) Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. Mol Immunol 40(13):921–929 [PubMed: 14725788]
17. Toh CH, Ticknor LO, Downey C, Giles AR, Paton RC, Wenstone R (2003) Early identification of sepsis and mortality risks through simple, rapid clot-waveform analysis. Implications of

lipoprotein-complexed C reactive protein formation. Intensive Care Med 29(1):55–61. doi: 10.1007/s00134-002-1557-2 [PubMed: 12528023]

18. Smith EY, Charles LA, Van Cott EM (2004) Biphasic activated partial thromboplastin time waveform and adverse events in nonintensive care unit patients. Am J Clin Pathol 121(1):138–141. doi:10.1309/W4F7-892W-JE6Y-1W7Y [PubMed: 14750251]
19. Penner JA (1998) Disseminated intravascular coagulation in patients with multiple organ failure of non-septic origin. Semin Thromb Hemost 24(1):45–52. doi:10.1055/s-2007-995822 [PubMed: 9515779]
20. Matsumoto T, Wada H, Nishioka Y, Nishio M, Abe Y, Nishioka J, Kamikura Y, Sase T, Kaneko T, Houdijk WP, Nobori T, Shiku H (2006) Frequency of abnormal biphasic aPTT clot waveforms in patients with underlying disorders associated with disseminated intravascular coagulation. Clin Appl Thromb Hemost 12(2):185–192 [PubMed: 16708120]
21. Toh CH, Samis J, Downey C, Walker J, Becker L, Brufatto N, Tejidor L, Jones G, Houdijk W, Giles A, Koschinsky M, Ticknor LO, Paton R, Wenstone R, Nesheim M (2002) Biphasic transmittance waveform in the APTT coagulation assay is due to the formation of a Ca(++)-dependent complex of C-reactive protein with very-low-density lipoprotein and is a novel marker of impending disseminated intravascular coagulation. Blood 100(7): 2522–2529. doi:10.1182/blood.V100.7.2522 [PubMed: 12239165]
22. Downey C, Kazmi R, Toh CH (1998) Early identification and prognostic implications in disseminated intravascular coagulation through transmittance waveform analysis. Thromb Haemost 80(1):65–69 [PubMed: 9684787]
23. Krause RD, Anand VD, Gruemer HD, Willke TA (1975) The impact of laboratory error on the normal range: a Bayesian model. Clin Chem 21(3):321–324 [PubMed: 1112041]
24. Hapke M, Patil K (1981) The establishment of normal limits for serum proteins measured by the rate nephelometer. Concepts of normality revisited. Hum Pathol 12(11):1011–1015 [PubMed: 7319488]
25. Stemerman MB (1985) Coagulation in the elderly. Clin Geriatr Med 1(4):869–885 [PubMed: 3830390]
26. La Bonte LR, Pavlov VI, Tan YS, Takahashi K, Takahashi M, Banda NK, Zou C, Fujita T, Stahl GL (2012) Mannose-binding lectin-associated serine protease-1 is a significant contributor to coagulation in a murine model of occlusive thrombosis. J Immunol 188(2):885–891. doi:10.4049/jimmunol.1102916 [PubMed: 22156595]
27. Thiel S, Vorup-Jensen T, Stover CM, Schwaeble W, Laursen SB, Poulsen K, Willis AC, Eggleton P, Hansen S, Holmskov U, Reid KB, Jensenius JC (1997) A second serine protease associated with mannan-binding lectin that activates complement. Nature 386(6624):506–510 [PubMed: 9087411]
28. Matsushita M (2009) Ficolins: complement-activating lectins involved in innate immunity. J Innate Immun 2(1):24–32. doi:10.1159/000228160 [PubMed: 20375620]
29. Takahashi K, Ezekowitz RA (2005) The role of the mannose-binding lectin in innate immunity. Clin Infect Dis 41(Suppl 7): S440–S444 [PubMed: 16237644]
30. Moller-Kristensen M, Hamblin MR, Thiel S, Jensenius JC, TakahashiK(2007)Burninjuryrevealsalteredphenotypeinmannanbindinglectin-deficientmice. J Invest Dermatol127(6):1524–1531
31. Chang WC, White MR, Moyo P, McClear S, Thiel S, Hartshorn KL, Takahashi K (2010) Lack of the pattern recognition molecule mannose-binding lectin increases susceptibility to influenza A virus infection. BMC Immunol 11(1):64. doi:10.1186/1471-2172-11-64 [PubMed: 21182784]
32. Larvie M, Shoup T, Chang WC, Chigweshe L, Hartshorn K, White MR, Stahl GL, Elmaleh DR, Takahashi K (2012) Mannose-binding lectin binds to amyloid beta protein and modulates inflammation. J Biomed Biotechnol 2012:929803. doi:10.1155/2012/929803 [PubMed: 22536027]
33. Ip WK, Lau YL, Chan SY, Mok CC, Chan D, Tong KK, Lau CS(2000) Mannose-binding lectin and rheumatoid arthritis in southern Chinese. Arthritis Rheum 43(8):1679–1687 [PubMed: 10943857]
34. Motomura W, Yoshizaki T, Ohtani K, Okumura T, Fukuda M, Fukuzawa J, Mori K, Jang SJ, Nomura N, Yoshida I, Suzuki Y, Kohgo Y, Wakamiya N (2008) Immunolocalization of a novel

collectin CL-K1 in murine tissues. J Histochem Cytochem 56(3): 243–252. doi:10.1369/jhc.7A7312.2007 [PubMed: 18040075]

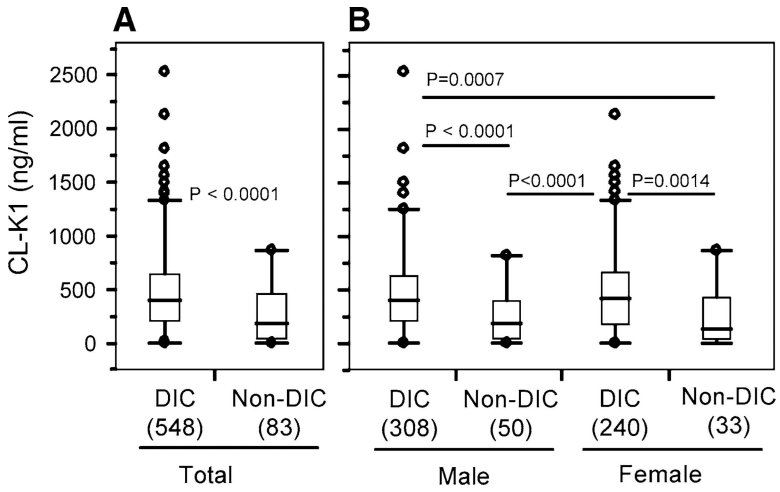

Fig. 1.

Elevated plasma CL-K1 levels in the DIC group compared with the non-DIC group. Plasma CL-K1 concentrations are plotted for DIC and non-DIC populations (**a**), and in these populations subdivided by male or female gender (**b**). Numbers in parentheses indicate specimen numbers. Statistical significance was analyzed by a nonparametric Wilcoxon/Kruskal–Wallis test and only *p* values less than 0.05 are indicated

Table 1

Patient demographics and diagnoses

	Group		*p* values[b]
	Non-DIC (*n* = 83)	**DIC (*n* = 549)**	
Age			
Median (IQ25–75 %)[a]	63.5 (50.5–73.3)	67.0 (53.0–78.0)	0.0886
Gender			
Female/Male (ratio)	33/50 (1/1.515)	240/309 (1/1.288)	0.4878
Diagnoses			
Neoplasm	18 (21.7)	123 (22.4)	1.000
Respiratory diseases	11 (13.3)	164 (29.9)	0.0014
Cardiac diseases	10 (12.1)	145 (26.4)	0.0038
Vascular diseases	15 (18.1)	130 (23.7)	0.3265
Coagulation disorders	9 (10.8)	102 (18.6)	0.0899
Gastrointestinal diseases	12 (14.5)	96 (17.5)	0.6387
Renal diseases	7 (8.4)	130 (23.7)	0.0009
Diabetic milieu	12 (14.5)	91 (16.6)	0.7502
Infection	17 (37.0)	322 (63.7)	0.0007

[a] *IQ* interquartile range

[b] Fisher's Exact Test, 2-tail

Table 2

Elevated plasma CL-K1 levels are associated with presence of DIC

	Median (IQ25–75 %)[a]	High/Total[b]	*p* values[c]	OR[d]	95 % CI[e]
Total					
Non-DIC	176 (33–463)	12/83 (14.5)			
DIC	409 (192 –657)	154/549 (28.1)	0.0074	1.929	1.041–3.866
Female					
Non-DIC	164 (47–517)	6/33 (18.2)			
DIC	417 (169–666)	71/240 (29.6)	0.2174	1.613	0.666–4.518
Male					
Non-DIC	186 (32–399)	6/50 (12.0)			
DIC	407 (201 –639)	83/309 (26.9)	0.0223	2.228	0.960–6.091

[a] Units are ng/ml. *IQ* indicates interquartile range

[b] "*High*" indicates number of patients with elevated plasma CL-K1 levels, defined as greater than 619 ng/ml; "*Total*" indicates total number of patients in the group. Numbers in parentheses indicate percent of those with elevated CL-K1 plasma levels

[c] Fisher's Exact Test

[d] Odds (OR) ratios were calculated by a nominal logistic regression model and adjusted to age

[e] *CI* confidence interval

Table 3

Plasma CL-K1 levels (ng/ml) by age cohort among patients with DIC

Age	Median (IQ25–75 %)[a]	High/Total (%)[b]
21–40	404 (243–631)	16/61 (26.3)
41–60	443 (206–715)	50/146 (34.3)
61–80	412 (155–648)	62/227 (27.3)
81–100	369 (194–579)	23/107 (21.5)

[a] *IQ* interquartile range

[b] "High" indicates number of patients with elevated plasma CL-K1 levels, defined as greater than 619 ng/ml; "Total" indicates total number of patients in the group. Numbers in parentheses indicate percent of those with elevated CL-K1 plasma levels. There was no statistical difference among different age groups (assessed by Likelihood Ratio test)

Table 4

Incidence of elevated plasma CL-K1 levels in subjects with other diseases, without or with DIC

Diagnosis	High/total (%)[a]		*p* values[b]
	Non-DIC	DIC	
Neoplasm	5/18 (27.8)	32/123 (26.1)	1.000
Respiratory diseases	1/11 (0.1)	54/164 (32.9)	0.1765
Cardiac diseases	1/9 (10)	39/145 (26.9)	0.4547
Vascular diseases	0/15 (0)	38/130 (29.2)	0.0116
Coagulation disorders	2/9 (22.2)	37/102 (36.3)	0.4897
Gastrointestinal diseases	3/12 (25.0)	33/96 (34.4)	0.7472
Renal diseases	1/7 (14.3)	41/130 (31.5)	0.4380
Diabetic milieu	0/12 (0)	23/91 (25.3)	0.0636
Infection	4/17 (23.5)	99/322 (30.8)	0.6017

[a]"High" indicates number of patients with elevated plasma CL-K1 levels, defined as greater than 619 ng/ml; "Total" indicates total number of patients in the group. Numbers in parentheses indicate percent of those with elevated CL-K1 plasma levels

[b]Wilcoxon/Kruskal-Wallis tests (Rank Sums)

Table 5

Incidence of elevated plasma CL-K1 levels by disease, independent of DIC

Diagnostics	High/Total (%)[a]		*p* values[b]
	Positive	Negative	
Neoplasm	37/141 (23.2)	129/491 (26.3)	1.000
Respiratory diseases	55/175 (31.4)	111/457 (24.3)	0.0070
Cardiac diseases	40/155 (25.8)	126/477 (26.4)	0.9167
Vascular diseases	38/145 (26.2)	128/487 (26.3)	1.000
Coagulation disorders	39/111 (35.1)	127/521 (24.4)	0.0238
Gastrointestinal diseases	36/108 (33.3)	130/524 (24.8)	0.0722
Renal diseases	42/137 (30.7)	124/495 (25.1)	0.1897
Diabetic milieu	23/103 (22.3)	143/529 (27.0)	0.3916
Infection	104/340 (30.6)	49/213 (23.0)	0.0633

[a] "High" indicates number of patients with elevated plasma CL-K1 levels, defined as greater than 619 ng/ml; "Total" indicates total number of patients in the group. Numbers in parentheses indicate percent of those with elevated CL-K1 plasma levels

[b] Fisher's Exact test, 2-tail