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Original Article

Elucidation of the Mechanism and Significance of the Erythrocyte Sedimentation Rate from Clinical Laboratory Data

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The erythrocyte sedimentation rate (ESR) is a widely used marker of inflammation, but the detailed mechanisms underlying the ESR remain unclear. We retrospectively collected laboratory data from our hospital's laboratory information system, and performed multiple linear regression analysis and correlation analysis to determine relationships between the ESR and other laboratory test parameters. The alpha-2, beta-2, and gamma fractions from serum protein electrophoresis, serum immunoglobulin (Ig) G, IgA, IgM, and complement C3 levels, plasma fibrinogen levels, and platelet count showed positive effects on the ESR; however, the serum albumin level showed negative effects. Since erythrocytes are negatively charged, an increase in positively charged proteins and a decrease in negatively charged albumin were suggested to increase the ESR. Notably, C-reactive protein (CRP) showed the third-strongest correlation with the ESR despite having no significant effect on the ESR. We also reviewed cases with discordant ESR and CRP levels to compare the disease profiles of high ESR/low CRP patients and low ESR/high CRP patients. The patients with high ESR/low CRP had a completely different disease profile from those with low ESR/high CRP. Since the ESR and CRP have different roles, they should be used as markers in a context-dependent manner.

Key words: complement, erythrocyte sedimentation rate, fibrinogen, immunoglobulin, serum protein electrophoresis

The erythrocyte sedimentation rate (ESR) is a widely used marker of inflammation. It has a long history of use, starting from the discovery of erythrocyte hyper-sedimentation as an indicator of poor physical status by early Greek physicians [1]. At the end of the 19th century, Biernacki, a Polish physician, found a correlation between the speed of erythrocyte sedimentation and the amount of fibrinogen in the blood, which led to the first clinical application of the ESR [2]. The first widely accepted ESR measurement method was established by Fahraeus and Westergren in 1921 [3,4], and ever since, ESR has been used as a marker in a variety of inflammatory diseases, especially rheumatism and collagen diseases. Some reports have suggested that C-reactive protein (CRP) is a more sensitive marker of inflammation than the ESR [5,6]. However, in some conditions, such as chronic infection, monoclonal gammopathy, and giant cell arteritis, the ESR is an indispensable marker for diagnosis and the assessment of disease activity [7-10]. Recently, a meta-analysis reported that the ESR is as sensitive and specific as CRP in the assessment of orthopedic inflammation [11].

The mechanisms that cause erythrocytes to descend in whole blood at different rates (*i.e.*, the mechanisms

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underlying the ESR) have been of interest, particularly in relation to serum or plasma proteins [12-16]. As mentioned above, fibrinogen is the blood protein that has been the longest studied in relation to ESR [17, 18]. Steinvil *et al.* previously suggested that serum fibrinogen levels may be the most important factor affecting the ESR, and they have also shown that hemoglobin levels negatively affect the ESR [19]. Although there have been studies that reported correlations between blood proteins, such as fibrinogen, and the ESR, most of them reported a simple correlation between the respective blood protein and the ESR. A detailed analysis of the complex mechanisms that determine the ESR will help clarify its clinical significance.

Large amounts of clinical laboratory data are stored in laboratory information systems, and the retrospective analysis of such data can provide important insights in laboratory medicine. In this study, we aimed to elucidate the effects of various factors on the ESR using data stored in our laboratory information system. We focused on the effects of serum and plasma proteins, including serum protein fractions obtained through electrophoresis, and the levels of albumin, immunoglobulins, complements, and plasma fibrinogen. In addition, we attempted to elucidate the specific context in which the ESR is superior to CRP as a marker of inflammation.

Materials and Methods

Data collection. The patient laboratory data and profiles were collected using a diagnostic support system (DSS; Abbott Japan, Tokyo) that is linked to our laboratory information system; this DSS allows researchers to extract laboratory datasets based on user-defined criteria. Patients younger than 18 years of age were excluded from the study.

ESR measurements. The ESR was measured by capillary photometric-kinetic method using a ROLLER 20 PN (Alifax, Polverara, Italy) that records only the 60-min ESR value.

Serum protein electrophoresis (serum protein fraction). Serum protein electrophoresis was performed by SRL (Tokyo) using capillary electrophoresis.

Measurements of blood cells. White blood cell (WBC), red blood cell (RBC), and platelet counts were determined through a combination of direct current detection and flow cytometry using an XN-9100 (Sysmex, Kobe, Japan).

Measurement of serum protein levels. Serum albumin levels were measured by a modified bromocresol purple assay using a LABOSPECT 008α (Hitachi High-Technologies, Tokyo). Plasma fibrinogen levels were measured according to the thrombin method using a STACIA (LSI Medience, Tokyo). The serum levels of complements C3 and C4, and immunoglobulin G (IgG), IgA, and IgM were all measured by the immunoturbidimetric method using the LABOSPECT 008α. Serum CRP levels were measured with the latex immunoturbidimetric method using the LABOSPECT 008α.

Statistical analysis. All statistical analyses were performed using the EZR statistics software (version 1.52) [20]. Multiple linear regression analysis was performed with the ESR as the dependent variable and the other factors as explanatory variables. Male and female sex were categorized as "1" and "2", respectively. Spearman's rank correlation analysis was also performed between the ESR and the other factors, and the strengths of the correlations were defined according to a previously used definition, namely: no correlation ($r \leq 0.1$), poor correlation ($0.1 < r < 0.3$), fair correlation ($0.3 \leq r < 0.6$), moderately strong correlation ($0.6 \leq r < 0.8$), and very strong correlation ($r \geq 0.8$) [21, 22]. Values of $p < 0.05$ were considered to indicate statistical significance.

Ethical approval. This study was conducted in accordance with the Declaration of Helsinki. The ethics committee of Nihon University Itabashi Hospital approved this study (approval no. RK-190212-5).

Results

Analysis 1: Multiple linear regression analysis, including serum protein electrophoresis. Serum protein electrophoresis is most suitable for observing the rough composition of blood proteins. Given that plasma fibrinogen cannot be detected by serum protein electrophoresis, data on both the serum protein fractions and plasma fibrinogen levels were collected as follows. The DSS apparatus detected 497 measurements in which the ESR, WBC count, RBC count, platelet count, serum total protein level, serum protein fractions by electrophoresis, and plasma fibrinogen level were simultaneously measured at Nihon University Itabashi Hospital between July 2016 and April 2021. To avoid the duplication of data, only the earliest measured data were included in the analysis for patients who

had undergone laboratory tests more than once. As a result, 486 patients (179 males and 307 females) with a median age of 59 years (range, 18 to 94 years) were selected. Among these 486 patients, 370 were outpatients and 116 were inpatients from the following departments: general medicine, 22 patients; rheumatology and immunology, 265 patients; neurology, 81 patients; hematology, 52 patients; nephrology, 35 patients; respiratory medicine, 11 patients; obstetrics and gynecology, 7 patients; gastroenterology, 4 patients; dermatology, 4 patients; neurosurgery, 3 patients; and ophthalmology, 2 patients. Serum protein fractions were converted into absolute amounts by multiplying the serum total protein concentration by the percentage of each fraction; for example, if the serum protein concentration was 6,000 mg/dl and the serum alpha-1 fraction was 5.3%, the amount of the serum alpha-1 fraction was calculated to be 318 mg/dl. Multivariate analysis was performed to elucidate the effects of serum protein fractions and plasma fibrinogen levels on the ESR. Multiple linear regression analysis was performed with ESR as the dependent variable and the other factors (age, sex, WBC count, RBC count, platelet count, albumin fraction, alpha-1 fraction, alpha-2 fraction, beta-1 fraction, beta-2 fraction, gamma fraction, and fibrinogen level) as explanatory variables.

The multiple linear regression analysis resulted in an optimized model consisting of 12 parameters (Table 1) with an adjusted R-squared value of 0.879 and a p-value < 0.001. Each of the variance inflation factors was less than 4, indicating that there were no serious issues concerning multicollinearity. The age, platelet count, alpha-2 fraction, beta-2 fraction, gamma fraction, and fibrinogen level showed significant t-values (> 2.00) and P-values (< 0.05), indicating that each of these parameters is an independent positive ESR determinant. The RBC count and albumin level also showed significant t- and p-values, indicating that they are independent negative ESR determinants.

Analysis 2: Multiple linear regression analysis focusing on individual protein levels. As shown in analysis 1, the serum albumin fraction, alpha-2 fraction, beta-2 fraction, and gamma fraction have significant effects on the ESR. We next tried to elucidate the effects of the components of individual serum protein fractions on the ESR. In most patients, the components of the alpha-2 fraction, such as the serum alpha-2 macroglobulin, haptoglobin, and ceruloplasmin levels, were not measured, and we were thus unable to analyze data on the components of the alpha-2 fraction. For the beta-2 fraction, we analyzed C3 and C4, and for the gamma fraction, we analyzed IgG, IgA, IgM, and CRP.

The DSS apparatus detected 209 consecutive measurements in which the ESR, WBC count, RBC count, platelet count, levels of serum albumin, C3, C4, IgG, IgA, IgM and CRP, and level of plasma fibrinogen were simultaneously measured at Nihon University Itabashi Hospital between July 2016 and April 2021. For

Table 1 Analysis 1: Results of multiple linear regression analysis of 12 factors, including serum protein electrophoresis data, for the erythrocyte sedimentation rate

	Median (range)	Estimated regression coefficient	Standard error	t-value	P-value	VIF
(Intercept)		−58.4	6.03	−9.70	< 0.001	
Age (years)	59 (18 to 94)	0.114	0.0259	4.40	< 0.001 †	1.27
Sex	179 males, 307 females	0.855	1.02	0.842	0.400	1.19
WBC (× 1,000 cells/μl)	6.20 (0.400 to 24.4)	−0.262	0.139	−1.88	0.0598	1.43
RBC (× 1,000 cells/μl)	4,110 (1,190 to 6,370)	−0.00343	0.000723	−4.74	< 0.001 †	1.72
Platelet (× 1,000 cells/μl)	240 (1.00 to 959)	0.0105	0.00446	2.35	0.0192 †	1.41
Albumin (mg/dl)	3,920 (1,550 to 5,370)	−0.00365	0.000994	−3.67	< 0.001 †	2.62
Alpha-1 (mg/dl)	309 (188 to 811)	−0.000873	0.000747	−0.117	0.907	3.91
Alpha-2 (mg/dl)	674 (307 to 1,450)	0.0536	0.00411	13.0	< 0.001 †	2.93
Beta-1 (mg/dl)	418 (185 to 980)	0.0154	0.00911	1.90	0.0585	1.73
Beta-2 (mg/dl)	367 (168 to 5,030)	0.0169	0.00163	10.4	< 0.001 †	1.33
Gamma (mg/dl)	1,250 (114 to 3,750)	0.0201	0.000917	21.9	< 0.001 †	1.06
Fibrinogen (mg/dl)	340 (73.0 to 830)	0.0990	0.00625	15.8	< 0.001 †	2.95

RBC, red blood cell; WBC, white blood cell; VIF, variance inflation factor; † indicates a statistically significant difference.

patients who had undergone laboratory tests more than once, only the earliest measured data were included in this analysis. In total, 200 patients (98 males and 102 females) with a median age of 60 years (range, 18 to 94 years) were selected. Among these 200 patients, 68 were outpatients and 132 were inpatients from the following departments: emergency medicine, 53 patients; general medicine, 23 patients; rheumatology and immunology, 61 patients; neurology, 11 patients; hematology, 9 patients; nephrology, 12 patients; respiratory medicine, 9 patients; cardiology, 13 patients; obstetrics and gynecology, 2 patients; gastroenterology, 2 patients; dermatology, 2 patients; and otorhinolaryngology, 3 patients.

Multiple linear regression analyses were performed with the ESR as the dependent variable and the other factors (age, sex, WBC count, RBC count, platelet count, and levels of albumin, fibrinogen, C3, C4, IgG, IgA, IgM, and CRP) as explanatory variables. These analyses resulted in an optimized model consisting of 13 parameters (Table 2) with an adjusted R-squared value of 0.813 and a p-value < 0.0001. Each of the variance inflation factors was less than 4, indicating that there were no serious issues concerning multicollinearity. The platelet count and levels of fibrinogen, C3, IgG, IgA, and IgM showed significant t-values (> 2.00) and p-values (< 0.05), indicating that they are independent positive ESR determinants. The serum albumin level also showed significant t- and p-values, indicating that it is an independent negative ESR determinant.

Analysis 3: Correlation analysis. We also performed Spearman's rank analysis of the correlations between the ESR and the other factors in analysis 2. In this test, the fibrinogen level demonstrated the highest correlation with the ESR ($r = 0.698$), indicating a moderately strong correlation. Seven factors (age, platelet count, and the levels of C3, C4, IgG, IgA, and CRP) were judged as having a fair correlation. The levels of albumin and IgM, and the RBC count showed poor correlations with the ESR, and the WBC count did not show any significant correlation with the ESR (Table 3).

Analysis 4: Analysis of cases with discordant ESR and CRP levels. For the 200 cases included in analy-

Table 3 Analysis 3: Results of Spearman's correlation analysis of 12 factors for the erythrocyte sedimentation rate

	r [Spearman]	Strength of linear relationship
Age	0.384 ($p < 0.001$)	Fair
WBC	0.0515 ($p = 0.469$)	None
RBC	−0.193 ($p = 0.00613$)	Poor
Platelet	0.388 ($p < 0.001$)	Fair
Albumin	−0.298 ($p < 0.001$)	Poor
C3	0.408 ($p < 0.001$)	Fair
C4	0.312 ($p < 0.001$)	Fair
IgG	0.539 ($p < 0.001$)	Fair
IgA	0.432 ($p < 0.001$)	Fair
IgM	0.166 ($p = 0.0189$)	Poor
CRP	0.517 ($p < 0.001$)	Fair
Fibrinogen	0.698 ($p < 0.001$)	Moderately strong

Table 2 Analysis 2: Results of multiple linear regression analysis of 13 factors for the erythrocyte sedimentation rate

	Median (range)	Estimated regression coefficient	Standard error	t-value	P-value	VIF
(Intercept)		−39.2	10.2	−3.86	< 0.001	
Age(years)	60 (18 to 94)	0.141	0.0580	2.44	0.0158	1.43
Sex	98 males, 102 females	3.58	2.20	1.63	0.105	1.26
WBC (× 1,000 cells/μl)	7.65 (0.500 to 27.6)	−0.489	0.270	−1.81	0.0722	1.60
RBC (× 1,000 cells/μl)	4,080 (1,380 to 5,970)	−0.000957	0.00152	−0.629	0.530	1.69
Platelet (× 1,000 cells/μl)	235 (2.00 to 658)	0.0323	0.0113	2.85	0.00486 †	1.59
Albumin (mg/dl)	3,500 (1,500 to 5,000)	−0.00765	0.00180	−4.26	< 0.001 †	2.05
C3 (mg/dl)	100 (30 to 200)	0.126	0.0576	2.19	0.0296 †	3.53
C4 (mg/dl)	25.0 (3.00 to 68.0)	0.0195	0.154	0.126	0.900	2.93
IgG (mg/dl)	1,190 (322 to 6,030)	0.0180	0.00175	10.3	< 0.001 †	1.61
IgA (mg/dl)	238 (62.0 to 1,730)	0.0398	0.00679	5.86	< 0.001 †	1.39
IgM (mg/dl)	84.0 (16.0 to 527)	0.0420	0.0154	2.73	0.00694 †	1.28
CRP (mg/dl)	1.07 (0.100 to 31.9)	0.396	0.253	1.57	0.119	2.42
Fibrinogen (mg/dl)	360 (106 to 830)	0.0919	0.0122	7.55	< 0.001 †	3.18

C3, complement C3; C4, complement C4; CRP, C-reactive protein; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; † indicates a statistically significant difference.

ses 2 and 3, we classified the ESR levels into tertiles as follows: ESR ≥ 47 mm/h was considered a high ESR level; 18 mm/h ≤ ESR ≤ 46 mm/h was considered a medium ESR level; and ESR ≤ 17 mm/h was considered a low ESR level. We also classified the CRP levels using tertiles as follows: CRP ≥ 3.19 mg/dL was considered a high CRP level; 0.30 mg/dl ≤ CRP ≤ 3.18 mg/dl was considered a medium CRP level; and CRP ≤ 0.29 mg/dl was considered a low CRP level. We next identified cases with discordant ESR and CRP levels, *i.e.*, cases with high ESR and low CRP levels (high ESR/low CRP group) or low ESR and high CRP levels (low ESR/high CRP group).

As shown in Table 4 and 5, 11 cases had high ESR/low CRP, and 6 cases had low ESR/high CRP. Nine of the 11 cases in the high ESR/low CRP group were patients with autoimmune diseases. Among them, 1 case (case no. 9) also had nontuberculous mycobacterial infection. The remaining 2 cases had nephrotic syndrome due to type II diabetes mellitus (case no. 5) or idiopathic interstitial pneumonia (case no. 6) (Table 4). Among the 6 cases in the low ESR/high CRP group, 5 patients had infectious diseases, and the remaining 1 case (case no. 2) had acute decompensated heart failure (Table 5). Factors that were identified to be independent determinants of the ESR in analysis 2 were also

Table 4 Analysis 4: Details of the cases with high ESR/low CRP

Case no.	Age (years)	Sex	ESR (mm/h)	CRP (mg/dl)	Platelet (× 1,000 cells/μl)	Albumin (mg/dl)	C3 (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	Fibrinogen (mg/dl)	Disease
1	77	Female	109	0.29	240	2.7	37	4,924	426	269	311	Sytemic lupus erythematosus
2	68	Female	105	0.14	10	3.7	75	6,034	426	153	261	Sjogren syndrome/chronic thyroiditis
3	57	Female	81	0.12	239	3.9	83	3,408	342	138	351	Systemic scleroderma
4	73	Female	65	0.1	218	3.9	99	1,456	137	72	390	Sjogren syndrome
5	59	Male	59	0.1	198	4.1	122	1,894	475	41	426	Nephrotic syndrome due to type II diabetes mellitus
6	75	Female	56	0.1	172	4.6	114	1,605	261	165	399	Idiopathic interstitial pneumonia
7	27	Female	53	0.1	497	4.0	130	2,318	234	202	195	Autoimmune hepatitis
8	68	Female	53	0.29	270	4.0	110	1,735	323	86	379	Dermatomyositis
9	71	Male	52	0.1	220	3.9	72	2,016	417	53	285	IgG4-related disease/nontuberculous mycobacteriosis
10	67	Female	49	0.13	198	3.3	37	2,171	351	89	322	Sytemic lupus erythematosus
11	48	Male	47	0.21	268	4.2	127	1,411	276	120	476	Systemic screloderma
		Average			230	3.8	91	2,634	333	126	345	

Table 5 Analysis 4: Details of the cases with low ESR/high CRP

Case no.	Age (years)	Sex	ESR (mm/h)	CRP (mg/dl)	Platelet (× 1,000 cells/μl)	Albumin (mg/dl)	C3 (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	Fibrinogen (mg/dl)	Disease
1	53	Male	14	7.79	128	1.8	39	371	125	41	208	Acute pericarditis due to viral infection
2	54	Male	2	7.06	39	3.3	117	1,012	98	132	332	Acute decompensated heart failure
3	76	Male	14	6.08	130	2.4	74	1,463	115	55	307	Sepsis
4	72	Female	10	5.95	183	2.7	76	1,647	160	35	350	Pyelonephritis
5	57	Female	15	5.47	72	2.4	66	836	203	79	170	Sepsis
6	61	Male	2	3.84	152	3.6	74	879	202	56	397	Pneumonia
		Average			117	2.7	74	1,035	151	66	294	

examined in the high ESR/low CRP group and high CRP/low ESR group (Tables 4 and 5). Comparisons of the average levels of these factors showed that the levels of IgG and IgA were more than two times higher, and the levels of platelets and IgM were almost two times higher in the high ESR/low CRP group than in the low ESR/high CRP group.

Discussion

In the present study, we performed two multivariate analyses and one correlation analysis to elucidate the factors that either affect or correlate with the ESR. According to the adjusted R-squared value, the linear regression model constructed in analysis 1 can explain 87.9% of the ESR values. The model in analysis 2 was able to explain 81.3% of the ESR values. Thus, our multivariate model was able to explain the mechanism of the ESR in detail, and it was found that blood proteins had a particularly large impact on the ESR.

Erythrocytes are negatively charged, and they repel one another. The presence of positively charged proteins is thought to increase the ESR [18]. In the present study, the beta-2 fraction containing C3 and the gamma fraction containing IgG, IgA, and IgM showed significant positive effects on the ESR [23-25]. Although fibrinogen cannot be detected by serum protein electrophoresis, it belongs to the beta-gamma region in plasma protein electrophoresis [26], and it also showed positive effects on the ESR. All of these fractions and proteins are positively charged, and thus they clearly increased the ESR [23-25]. In contrast, albumin is negatively charged [23-25], and it showed a significantly negative effect on the ESR in both analyses 1 and 2; this was in accord with a previous report [27]. Taking these findings together, the charge on blood proteins appears to have an essential role in determining the ESR. Although it has been considered that fibrinogen makes a greater contribution to the ESR than other proteins [1,17,18], the results of the present study suggest that the contributions of other proteins are also significant. Several previous reports have described that fibrinogen and albumin are important determinants of the ESR [17-19,27], and in the present study, we were able to determine the mechanism of the ESR in more detail by adding serum protein fractions, immunoglobulins, and complements to the multivariate analysis.

In the present study, the alpha-2 fraction also showed a significant positive effect on the ESR. Due to insufficient data on the components of the alpha-2 fraction, such as alpha-2-macroglobulin, haptoglobin, and ceruloplasmin, a detailed analysis of the alpha-2 fraction was not possible in analysis 3. The alpha-2 fraction does not show a distinct charge like the albumin, beta-2, or gamma fractions [23-25]. Thus, the impact of the alpha-2 fraction on the ESR indicates that the electrostatic charge alone does not determine the ESR. Among the alpha-2 fraction proteins, haptoglobin and ceruloplasmin are known to be representative acute-phase proteins [26]. Increases in the serum levels of these proteins might have contributed to an increase in the ESR. Additionally, in Table 4, 1 case (case no. 5, which had nephrotic syndrome due to type II diabetes mellitus) had high ESR/low CRP. Since the alpha-2 fraction is increased in nephrotic syndrome [28], the high ESR in this case may have been due to the increased alpha-2 fraction.

As for analysis 3, all eight blood proteins showed a poor to moderately strong correlation with the ESR. It is worth noting that CRP showed the third-strongest correlation with the ESR in analysis 3 despite having no significant effect on the ESR in analysis 2. CRP belongs to the serum gamma fraction and it has a negative charge [26]. The negative charge of CRP did not significantly affect the ESR in the multivariate analysis, likely because CRP is much less abundant than other blood proteins. Thus, although the ESR and CRP are both markers of inflammation and correlate reasonably well, they are poorly related to each other directly. In other words, the ESR and CRP may have different roles as inflammatory markers, because the ESR is much less influenced by CRP than by other proteins in the blood.

As shown in analysis 4 (Tables 4 and 5), the disease profiles of the high ESR/low CRP group and the low ESR/high CRP group were significantly different. The high ESR/low CRP group mostly consisted of patients with autoimmune diseases. In contrast, the low ESR/high CRP group was dominated by patients with acute inflammation, and acute infections accounted for the majority of the cases. This result is consistent with other previous reports, and it also indicated that the discordance between the ESR and CRP has diagnostic value [29,30]. Although most of the cases with a high ESR in this study had autoimmune diseases, the ESR may also play a significant role in chronic infections and mono-

clonal gammopathy [7-10,29,30]. As shown in Tables 4 and 5, the average levels of IgG and IgA were more than two times higher, and those of platelets and IgM were almost two times as high in the high ESR/low CRP group than in the low ESR/high CRP group. This suggests that IgG, IgA, IgM, and platelets, the levels of which are elevated in autoimmune diseases, chronic infections, and monoclonal gammopathy, have a central role in the elevation of the ESR. On the other hand, in acute-phase reactions, the CRP level increases within 1 to 2 days, whereas the C3 level increases after 4 to 6 days, and the fibrinogen level increases after 1 to 2 weeks [28]. Therefore, although increases in C3 and fibrinogen lead to an increase in the ESR, these increases occur later than the increase in the CRP, resulting in the discordance between the ESR and CRP level. Taken together, these data show that the ESR and CRP have different roles, and they should thus be used as markers in a context-dependent manner.

A relationship between the platelet count and the ESR has been shown in previous reports [31,32]. Since platelets are negatively charged [33], it is assumed that they repel the negative charge of erythrocytes. However, in the present study, the platelets showed a positive effect on the ESR in both analyses 1 and 2, and a fair positive correlation with the ESR in analysis 3. Therefore, the charge of the platelets is not sufficient to explain the mechanism by which platelets increase the ESR, and further studies are required.

The present study has several limitations. First, both the age and RBC count had a significant effect on the ESR in analysis 1, but not in analysis 2. This discrepancy may have been due to the differences in the patient populations in the two analyses. In particular, the distribution of patients among departments differed greatly between the patient populations of analyses 1 and 2. In addition, it is possible that attending physicians may have decided upon different tests for people of different age groups and those with different RBC counts, which may have resulted in the different populations in analyses 1 and 2. There have been several reports on the relationship between age and ESR [34-36]. The RBC count is proportional to the hemoglobin concentration, and the hemoglobin concentration has been reported to have a negative effect on the ESR [14,19]. In the future, it will be necessary to verify whether the RBC count and the hemoglobin concentration really have a significant impact on the ESR. In the present study, we used the RBC count rather than the hemoglobin concentration or hematocrit to retain consistency with the WBC and platelet counts. However, the importance of the effect of the hematocrit on the ESR has previously been reported [37]. In the present study, we also constructed a multiple regression model by replacing the RBC count with the hematocrit for analyses 1 and 2. In analysis 1, a slight decrease to 0.873 was seen in the adjusted R-squared value, and the *t*-value and *p*-value of the hematocrit were 0.540 and 0.589, respectively, with no significant effect on the ESR. On the other hand, in analysis 2, the adjusted R-squared value was the same (0.813), but the *t*-value of the hematocrit was -0.498 and the *p*-value was 0.619, which indicated no significant effect on the ESR (data not shown). We cannot conclude whether the RBC count or hematocrit should be used as an indicator of the ESR, but considering the results of analysis 1, the RBC count may be superior. In addition, due to multicollinearity issues, it was not possible to perform multiple linear regression analysis using the RBC count and hematocrit at the same time.

Secondly, there is the issue of the ESR measurement method itself. Originally, the gold standard for ESR measurement was the Westergren method [38,39]. The sedimentation reaction consists of three stages: the rouleaux formation reaction, in which erythrocytes aggregate (the first 10 min); sedimentation (the next 40 min); and cell packing (the last 10 min). While the Westergren method measures the overall length of the sedimentation reaction for these three stages, the capillary photometric-kinetic method, which we are currently employing in our clinical laboratory, is an alternative method that mainly measures rouleaux formation. Alternative methods, including the capillary photometric-kinetic method, require less time to produce results, and are now commonly used in laboratories around the world [39]. However, some differences with the Westergren method have been described [40-42]. Kim *et al.* reported that in monoclonal gammopathy, the ESR may be less likely to be elevated when measured by the capillary photometric-kinetic method than when measured by the Westergren method [43]. This indicates that the ESR determined by the capillary photometric-kinetic method is less susceptible to immunoglobulins. On the other hand, Cha *et al.* analyzed the correlations between the capillary photometric-kinetic or Westergren method and plasma

protein fractions, and reported that the capillary photometric-kinetic method showed a stronger correlation with the alpha-1, alpha-2, beta-2, and gamma fractions than did the Westergren method [15]. Thus, since the relationships between the ESR and blood proteins are affected by differences in the ESR measurement methods, our results may have been different had the Westergren method been used.

In summary, the positive charge of the beta-2 fraction (C3), gamma fraction (IgG, IgA, and IgM), and fibrinogen had distinct positive effects on the ESR and showed correlations with the ESR in this retrospective analysis of data from a laboratory information system. Also, the negative charge of albumin showed a negative effect and correlation with the ESR. Thus, the electrostatic characteristics of serum or plasma proteins have a critical effect on the ESR. However, the alpha-2 fraction and platelet levels were also suggested to affect the ESR, indicating that the electrostatic characteristics are not the only factors affecting the ESR. Moreover, the ESR has a distinct role from that of CRP, and is considered to be an important inflammatory marker.

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