RESEARCH ARTICLE

The analysis of false prolongation of the activated partial thromboplastin time (activator: silica): Interference of C-reactive protein

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Background: To investigate the effect of C-reactive protein on the activated partial thromboplastin time (APTT) (different activators) in different detecting systems.

Methods: The C-reactive protein and coagulation test of 112 patients with the infectious disease were determined by automation protein analyzer IMMAG 800 and automation coagulation analyzer STA-R Evolution, respectively. The pooled plasma APTT with different concentrations of C-reactive protein was measured by different detecting system: STA-R Evolution (activator: silica, kaolin), Sysmex CS-2000i (activator: ellagic acid), and ACL TOP 700 (activator: colloidal silica). In addition, the self-made platelet lysate (phospholipid) was added to correct the APTT prolonged by C-reactive protein (150 mg/L) on STA-R Evolution (activator: silica) system.

Results: The good correlation between C-reactive protein and APTT was found on the STA-R Evolution (activator: silica) system. The APTT on the STA-R Evolution (activator: silica) system was prolonged by 24.6 second, along with increasing C-reactive protein concentration. And the APTT of plasma containing 150 mg/L C-reactive protein was shortened by 3.4-6.9 second when the plasma was mixed with self-made platelet lysate. However, the APTT was prolonged unobviously on other detecting systems including STA-R Evolution (activator: kaolin), Sysmex CS-2000i, and ACL TOP 700.

Conclusion: C-reactive protein interferes with the detection of APTT, especially in STA-R Evolution (activator: silica) system. The increasing in C-reactive protein results in a false prolongation of the APTT (activator: silica), and it is most likely that C-reactive protein interferes the coagulable factor binding of phospholipid.

KEYWORDS

activated partial thromboplastin time, C-reactive protein, detecting system

1 | INTRODUCTION

Activated partial thromboplastin time (APTT), a sensitive screening experiment in intrinsic coagulation system, is widely used to monitor preoperative bleeding and heparin therapy. C-reactive protein (CRP) is a classical acute phase reaction protein, which could elevate in inflammation, malignant tumor, ischemia, damage of tissue,

and so on. In clinical, we found partial patients with higher CRP and had a distinct prolonged APTT, especially those got infection and injury excepting congenital or acquired clotting protein deficiency. Therefore, our study investigates the impact of C-reactive protein on APTT (different activators) in various detecting systems, and proceeds with analyzing of correcting test by means of self-made platelet lysate (phospholipid).

2 | MATERIAL AND METHODS

2.1 | Subjects

There were 112 patients (51 females and 61 males, age range: 4-76 ages, median age: 45), excluding having history of liver and kidney disease, recent medication, and bleeding who were admitted to the second Affiliated Hospital and Yuying children's Hospital of Wenzhou Medical University from December 2015 to March 2016. Of these patients, the serum CRP, activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (FIB), and thrombin time (TT) were detected by automation protein analyzer IMMAG 800 and automation coagulator STA-R Evolution, respectively. This study was approved by the hospital ethics committee, and informed consents were obtained from patients or children's guardians.

2.2 | Preparation of pool plasma

2.7 milliliter vein blood of 10 healthy individuals (5 females and 5 males, CRP < 8 mg/L) was collected into vacuum tubes containing 0.3 millimeter 0.109 M sodium citrate. The samples were obtained after $1500 \times g$ 10 minutes centrifugation. Pool plasma is composed of 10 equal portion healthy plasma and stored at -80°C until analysis.

2.3 | Instruments and reagents

Automation coagulator STA-R Evolution as well as its APTT reagent: STA-PTT A kit (activator: silica, lot number: 113984) and C.K.PREST[®] (activator: kaolin, lot number: 112327) (Diagnostica Stago, France), automation coagulator ACL Top 700 as well as its APTT reagent (activator: colloidal silica, lot number: NO657295) (Beckman Coulter, USA), and automated coagulator CS-2000i as well as its APTT reagent (activator: ellagic acid, lot number: 557144) (Sysmex, Japan) were used to measure the APTT of different pool plasma. Purified CRP (lot number: C140901, 3×10^3 mg/L, Rongbai biological technology Company, Shanghai, China) was used as interference reagent. Those CRPs were detected by Protein Analyzer IMMAG 800 as well as its CRP reagents (lot number: M505776, Beckman Coulter, USA). Self-made phospholipids (the lysate of thrombocytes) were obtained from platelet of 10 healthy individuals.

2.4 | Preparation of phospholipid (the lysate of thrombocytes)

Blood from 10 healthy individuals was drawn into vacuum tube containing 0.109 M sodium citrate, then centrifuging at $200 \times g$ 10 minutes for 2 times and isolating platelet-rich plasma (PRP).¹ The PRP was centrifuged at $1500 \times g$ 10 minutes under 4°C to get sediments that contain enrichment platelet, and the pellet in Eppendorf Tube was repeatedly refrozen and thawed more than 5 times manually, and stored at -80°C until analysis.

2.5 | Experimental methods

The serums of 112 patients were measured by an immunoturbidimetric assay on IMMAG 800, and the APTT, PT, FIB, and TT were detected on automation coagulator STA-R Evolution (activator: silica). Purified CRP was added into the pool plasma, and gained terminal concentrations ranking 0.0 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, 250 mg/L, 300 mg/L. According to manufacturer's instructions, the APTT of plasma containing various CRP concentrations was detected by STA-R Evolution (two activators: silica, kaolin), ACL Top 700 and CS-2000i, respectively. The self-made phospholipids were diluted 2, 5, 10, 15, and 20 times with pool plasma, respectively. Equal volume lysate of thrombocytes diluted different folds was mixed with the plasma which CRP concentration was 150 mg/L, respectively. The APTT above mixtures was analyzed by STA-R Evolution (activator: silica).

2.6 | Statistics analysis

Statistical analysis was performed by SPSS 22.0 software. The relation between APTT and CRP was analyzed by linear regression analysis and *t* test. *P* < .05 was considered as statistical significance.

3 | RESULTS

3.1 | The relationship between CRP and APTT (activator: silica)

Along with increasing CRP concentrations, APTT detected on STA-R Evolution (activator: silica) had a tendency to extend, while PT, FIB, and TT had no obvious change in 112 patients involved in this study (Figure 1).

3.2 | The pooled plasma APTTs measured in the four systems

The four different systems were used to detect APTT of the same original plasma orderly. Surprisingly, APTTs were 34.5 second, 30.7

FIGURE 1 Correlation between CRP and APTT (activator: silica) among the 112 patients

TABLE 1 Different APTT on four different detecting systems

Systems	STA-R: silica	STA-R: kaolin	CS-2000i: ellagic acid	ACL TOP 700: colloidal silica
APTT (s)	34.5 ± 1.5	30.7 ± 1.4	30.6 ± 0.5	37.7 ± 0.8

FIGURE 2 Correlation between CRP and APTT in different detecting systems. (◆: STA-R Evolution (activator: silica), linear regression equation $y = 0.0882x + 34.769$, $R^2 = 0.9759$, $P < .005$; ■: STA-R Evolution (activator: kaolin), linear regression equation $y = 0.0035x + 36.847$, $R^2 = 0.3024$, $P = .003$; ▲: CS-2000i (activator: ellagic acid), linear regression equation $y = 0.0164x + 30.656$, $R^2 = 0.9801$, $P < .005$; ×: ACL Top 700 (activator: colloidal silica), linear regression equation $y = 0.0044x + 30.105$, $R^2 = 0.724$, $P = .125$)

second, 30.6 second and 37.7 second, which were significantly different (Table 1) in STA-R: silica, STA-R: kaolin, CS-2000i, ACL TOP 700 systems, respectively.

The pooled plasma APTTs with different density of CRP were measured by the four different detecting systems, respectively. As shown in Figure 2, APTTs, to varying degrees, were positively correlated with the concentration of CRP. APTTs detected by STA-R Evolution (activator: silica) significantly increase with the increasing concentration of CRP ($R^2 = 0.98$, $P < .005$), but APTT in the rest of systems had no significant change: STA-R Evolution (activator: kaolin) ($R^2 = 0.30$, $P < .005$), ACL Top 700 ($R^2 = 0.72$, $P = .125$), and CS-2000i ($R^2 = 0.98$, $P < .005$).

3.3 | Phospholipids corrected the prolonged APTT

Subsequently, to verify whether CRPs interfere with the phospholipid that come from ATPP reagents, different dilution ratio of the lysate of thrombocyte (self-made phospholipids) was added into the mixed plasma to correct APTT which prolonged by C-reactive protein (150 mg/L) on STA-R Evolution (activator: silica; Table 2). As shown in Figure 3, the APTTs of the plasma shortened by 3.4–6.9 second.

4 | DISCUSSION

APTT is one of the pivotal screening tests in intrinsic coagulation system. Prolonged APTT may indicate use of heparin, abnormal liver function, coagulation factors deficiency, medicine, false reagents, antiphospholipid antibody, etc. In clinical,^{2,3} however, we found that APTT prolonged in some patients who got mild to serious infection and traumatism excluding some factors like congenital or acquired clotting protein deficiencies and antiphospholipid antibody

syndrome, false samples, and agents, and patients had no bleeding tendency. Those patients had increasing CRP in serum, so false prolonged APTT may be related with the increasing CRP. To understand the relationship between APTT and CRP, we analyzed the relation of CRP level and APTT in the 112 unrelated patients and found that APTT (activator: silica) had a prolongation tendency with increasing CRP levels ($y = 0.0613x + 42.647$, $R^2 = 0.2372$, $P < .005$).

To further discuss that CRP has an impact on APTT, the pooled plasma APTT with different concentrations of CRP was measured by four different detecting systems, respectively. Interestingly, APTT (activator: silica) had an obviously prolongation along with the increasing concentrations of CRP: APTT prolonged by 24.6 second when CRP was 300 mg/L ($y = 0.0882x + 34.769$, $R^2 = 0.9759$, $P < .005$), although the data from the other three detecting system change unobviously. Among them, APTT on CS-2000i and STA-R Evolution (activator: kaolin) and ACL Top 700 system prolonged by 5 second at most, 0.5 second, 1.0 second, respectively. Different detecting systems have different methodology,⁴ and the mixed plasma's APTT without any purified CRP was 34.5 second, 30.7 second, 30.6 second in STA-R Evolution (activator: silica), STA-R Evolution (activator: kaolin), ACL Top 700, and CS-2000i, respectively.

CRP is homozygous annular pentameric protein containing 5 same deglycosylated polypeptide subunits.⁵ It involves in the non-specific immune response not only interact with polysaccharides existing in various cells, fungi, and protozoan but also have high affinity to phospholipids.⁶ Subsequently, CRP binds with nucleic acid and membrane phosphatidylcholine in the presence of calcium. On the structure of phosphatidic acid, both phosphatidylcholine and cephalin are divided into different groups. One of the composition of APTT reagents is cephalin (substitution of platelet factor 3). So the CRP may interact with cephalin in APTT reagent, and it results in the

TABLE 2 Different APTT after add different dilution ratio of phospholipid measured on STA-R Evolution (activator: silica)

APTT (s)	Dilution ratio of phospholipid					
	1	2	5	10	15	20
Before	50.9					
After	47.5	45.2	44.4	45.6	45.1	44.0

FIGURE 3 The lysate of thrombocyte partially shortened the APTT of plasma containing 150 mg/L CRP on STA-R Evolution (activator: silica)

prolongation of APTT. Cheng Hock Toh et al.⁷ reported that biphasic transmittance waveform of APTT is due to the calcium-dependent precipitation of a complex of very low-density lipoprotein (VLDL) and CRP. Moreover, disseminated intravascular coagulation (DIC) patients often have high CRP levels, and the biphasic waveform to early predict impending DIC was obtained from the MDA 180 automated coagulation analyzer. In the present study, we considered that the reason why APTT (activator: silica) had an apparent characteristic along with the increasing CRP levels may be that CRP disturbed the activity of phospholipid (cephalin) in APTT reagents. Therefore, to verify CRP bind with the cephalin, we added the lysate of thrombocyte (self-made phospholipids) to the plasma containing 150 mg/L CRP and then measured its APTT (activator: silica) on STA-R Evolution coagulation analyzer. And it was found that the mixed plasma APTTs detected by STA-R Evolution system shortened by 3.4–6.9 second, which indicated that CRP may interfere with the activity of phospholipid. However, the self-made phospholipid failed to shorten the normal APTT. We guessed this may attribute to its concentrations, purity, human source, and APTT reagents which cephalin commonly extracted from rabbit cerebral tissues or synthetic phospholipids. Indeed, these apparently conflicting findings may stem from phospholipids differences or different affinity of CRP.

Our study showed that CRP level has a non-negligible effect on the plasma APTT in different detecting systems. Particularly, the effect of CRP on APTT varies from different activator (silica and kaolin) and detecting methods. Therefore, we conjecture that the different activator of coagulating factor 12 (F12) has different activating way, which may be result in activable F12 binding

different sites of phospholipid. Further study will be performed in future.

In summary, this study revealed high-level CRP influence with APTT in different automation coagulator systems, particularly in STA-R Evolution (activator: silica). Two or more systems should be highly recommended in the clinical laboratory, and the clinician should make a correct management according to a comprehensive assessment of patients.

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