

Platelets and Sera from Donors of Convalescent Plasma after Mild COVID-19 Show No Procoagulant Phenotype

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Abstract

Coronavirus disease-2019 (COVID-19) is associated with increased thromboembolic complications. Long-term alteration in the coagulation system after acute COVID-19 infection is still a subject of research. Furthermore, the effect of sera from convalescent subjects on platelets is not known. In this study, we investigated platelet phenotype, coagulation, and fibrinolysis in COVID-19 convalescent plasma (CCP) donors and analyzed convalescent sera-induced effects on platelets. We investigated CCP donors who had a history of mild COVID-19 infection and donors who did not have COVID-19 were used as controls. We analyzed phosphatidylserine (PS) externalization, CD62p expression, and glycoprotein VI (GPVI) shedding both in platelet-rich plasma (PRP) and after incubation of washed healthy platelets with donors' sera using flow cytometry. Coagulation and fibrinolysis systems were assessed with thromboelastometry. Forty-seven CCP donors (22 males, 25 females; mean age (\pm SD): 41.4 ± 13.7 years) with a history of mild COVID-19 infection were included. Median duration after acute COVID-19 infection was 97 days (range, 34–401). We did not find an increased PS externalization, CD62p expression, or GPVI shedding in platelets from CCP donors. Sera from CCP donors did not induce PS externalization or GPVI shedding in healthy platelets. Sera-induced CD62p expression was slightly, albeit statistically significantly, lower in CCP donors than in plasma donors without a history of COVID-19. One patient showed increased maximum clot firmness and prolonged lysis time in thromboelastometry. Our findings suggest that procoagulant platelet phenotype is not present after mild COVID-19. Furthermore, CCP sera do not affect the activation status of platelets.

Keywords

- ▶ procoagulant platelets
- ▶ platelet activation
- ▶ convalescent plasma
- ▶ COVID-19

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Introduction

Thromboembolic complications are common in patients suffering from severe coronavirus disease-2019 (COVID-19).^{1,2} Various organs are affected by micro and macro thrombosis as a result of COVID-19-induced coagulopathy.^{3,4} Thrombotic complications can lead to multiorgan failure and mortality in severe cases.⁵ Both cellular and plasma elements of the coagulation system show abnormalities in COVID-19.⁶ Platelets contribute not only to the hypercoagulable state in COVID-19 patients but also to the systemic inflammatory response (cytokine storm) by releasing inflammatory mediators.⁷ The expression of P-selectin and CD63 is correlated with D-dimer in severe COVID-19 patients, suggesting an association between platelet activation and COVID-19-associated coagulopathy.⁸ We have previously shown that platelets of COVID-19 patients express a procoagulant phenotype.^{9,10} Furthermore, a correlation between procoagulant platelets and thrombosis as well as mortality has been shown in COVID-19 patients.^{10,11} As more people recover from COVID-19 infection and continue to experience symptoms,¹² discussion has begun over the possibility of persistent coagulopathy even after the acute infection period.^{13–15}

COVID-19 convalescent plasma (CCP) is used in the treatment of COVID-19.^{16–18} However, concerns have been expressed whether plasma components in CCP can shift the already imbalanced coagulation system to a more hypercoagulable state.¹⁶ Furthermore, we have previously shown that immunoglobulin G fractions from severe COVID-19 patients induce a procoagulant phenotype in healthy platelets.¹⁰ To our best knowledge, the effect of CCP on platelet phenotype and activation has not been investigated earlier.

The aims of this study were (1) to investigate the procoagulant platelet phenotype and platelet activation after acute infection in COVID-19 patients, (2) to investigate the effect of CCP on healthy platelets, (3) to measure the viscoelastic properties of blood using a rotational thromboelastometry in CCP donors.

Methods

Study Cohort

This study was conducted between January 2021 and July 2021. Plasma donors who had a mild COVID-19 infection at least 4 weeks before plasma donation were invited to participate in the study (CCP donors). Donors who were hospitalized for COVID-19 infection or who did not have a positive SARS-CoV-2 polymerase chain reaction test or SARS-CoV-2 antibody enzyme immunoassay were excluded from the study. Information on medical history and COVID-19 infection was obtained with a questionnaire. Blood samples were collected before plasma donation and either tested immediately or frozen for later analysis. Additionally, citrated blood samples and native blood samples were also collected from plasma donors who did not have a COVID-19 infection to establish control values for flow cytometry (FC) measurements. Written informed consent was obtained from all study participants.

Assessment of Platelet Phenotype

Platelets were isolated from citrated blood of the healthy plasma donors and/or CCP donors and tested within 3 hours. In brief, whole blood was centrifuged (120 g, 20 minutes [min*] at room temperature [RT], without brakes), and PRP was gently separated and used for further analysis. Where indicated, PRP was incubated with buffer or thrombin receptor activating peptide (TRAP-6, 2.5 and 10 µM; Hart Biologicals, Hartlepool, UK) for 15 minutes at RT. Platelets were then stained with annexin V-FITC and CD62p-APC (ImmunoTools, Friesoythe, Germany) and directly analyzed by FC. Test results were determined as fold increase (FI) of the percentage of double phosphatidylserine (PS)/CD62p-positive events in platelets.

For the assessment of GPVI shedding, platelets were stained with 1 µL of phycoerythrin (PE)-labeled anti-GPVI monoclonal antibodies (BD, San Jose, CA) for 15 minutes at RT in the dark and analyzed by FC. Where indicated, platelets treated with collagen-related peptide (CRP, 2.5 µg/mL) (CambCol laboratories, Ely, UK) served as positive control. Changes in GPVI expression on the platelet surface were quantified as percentage of reduction in the GPVI-positive platelet population and normalized to baseline.

Investigation of Antibody-Mediated Effects on Platelets

The ability of sera to induce procoagulant platelets was determined by incubating the sera from healthy controls, plasma donors, and CCP donors with washed platelets from blood donors. Platelets were obtained from blood donors, whose platelets are known to have a good response in the heparin-induced platelet activation assay. Each sample was tested with platelets from one donor.

Prior to use, all sera samples were heat-inactivated at 56 °C for 30 minutes, followed by a sharp centrifugation step at 5,000 g. The supernatant was collected in a fresh tube. For the determination of procoagulant platelets, 5 µL serum was incubated with 25 µL washed platelets (7.5×10^6) for 1 hour under rotating conditions at RT. Platelets co-incubated with TRAP-6 (10 µM; Hart Biologicals, Hartlepool, UK) and ionomycin (5 µM, 15 minutes* at RT [Sigma-Aldrich, St. Louis, MO]) were used as a positive control for procoagulant platelets. Afterwards, samples were washed once (7 minutes, 650 g, RT, without brake) and gently resuspended in 75 µL of phosphate-buffered saline (PBS; Biochrom, Berlin, Germany). Platelets were then stained with annexin V-FITC and CD62-APC (ImmunoTools, Friesoythe, Germany) and directly analyzed by FC. Test results were determined as FI of the percentage of double PS/CD62p-positive events in platelets upon incubation with donors' sera compared with healthy controls.

For the determination of GPVI shedding, aforementioned sera-treated washed platelets were stained with 1 µL of PE-labeled anti-GPVI (BD for 15 minutes at RT in the dark. After incubation, platelets were filled up with PBS to a final volume of 500 µL and immediately assessed by FC. Platelets incubated with TRAP-6 (10 µM; Hart Biologicals, Hartlepool, UK) and ionomycin (5 µM, 15 minutes at RT [Sigma-Aldrich, St. Louis, MO]) were used as a positive control. Changes in GPVI

expression on the platelet surface were quantified as percentage of reduction in the GPVI-positive platelet population, and normalized to washed platelets that were treated with sera from healthy controls.

Thromboelastographic Assays

Citrated blood samples from CCP donors were analyzed within 2 hours using a viscoelastic test system (ClotPro; Enicor GmbH, Munich, Germany). Blood coagulation is determined by elastic motion (clockwise and anticlockwise) of a cylindrical cup including blood mixed with activator reagents around a fixed pin. The motion of the cup is recorded and the data are converted into thromboelastographic amplitude values that are plotted over time. The rotation of the cup is progressively reduced depending on the elastic properties of the formed clot. We used the extrinsic assay (EX test), fibrinogen assay (FIB test), and the tissue plasminogen activator assay (tPA test) according to the manufacturer's instructions. In brief, in the EX test, clotting is triggered by tissue factor. This test appears to be sensitive to anticoagulation, fibrinogen, factor XIII, and hyperfibrinolysis. In the FIB test, platelets are inhibited by cytochalasin D and a synthetic GP2b3a antagonist. The FIB test indicates fibrinogen levels and fibrin polymerization in citrated blood. The tPA test is similar to the EX test but contains an additional 650 to 700 ng/mL of recombinant tPA (r-tPA), an activator of plasmin, to determine fibrinolysis resistance. The following parameters were estimated during the study: clotting time, maximum clot firmness, lysis time (time from the beginning of the clot formation until 50% of clot lysis), and maximum clot lysis. The normal range specified by the manufacturer was used in all measured parameters.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to any study-related procedure. The study protocol was approved by the Institutional Review Board of the University of Tübingen.

Data Sharing Statement

Data may be requested for academic collaboration from the corresponding author.

Statistics

Statistical analyses were performed using GraphPad Prism 7 (La Jolla, CA). *t*-Test was used to analyze normally distributed results. Nonparametric test (Mann-Whitney test) was used when data failed to follow a normal distribution as assessed by the D'Agostino-Pearson omnibus normality test. A *p*-value of <0.05 was assumed to represent statistical significance.

Results

Study Cohort

Forty-nine CCP donors were included in the study. Two donors were excluded later because of the negative SARS-

Table 1 Demographic and clinical characteristics of the CCP donors

| Demographic data | |
|---|-------------|
| Age (years) | 41.4 ± 13.7 |
| Sex | |
| Female | 25 (53%) |
| Male | 22 (47%) |
| SARS-CoV-2 infection | |
| Duration of infection (days) | 8.6 ± 6.5 |
| Symptoms | |
| Fever | 24 (51%) |
| Anosmia | 25 (53%) |
| Taste disorder | 20 (43%) |
| Eyes (redness, inflammation) | 3 (6%) |
| Headache | 33 (70%) |
| Sore throat | 18 (38%) |
| Congestion or runny nose | 26 (55%) |
| Cough | 27 (57%) |
| Shortness of breath or difficulty breathing | 18 (38%) |
| Pneumonia | 0 (0) |
| Nausea/vomiting/diarrhea | 5 (11%) |
| Fatigue | 37 (79%) |
| Limb/joint/back pain | 27 (57%) |
| Skin rash | 1 (2%) |
| Time to blood collection after the acute infection (days) | 97 (34–401) |
| Comorbidities | |
| Arterial hypertension | 5 (11%) |
| Diabetes mellitus type II | 2 (4%) |
| Asthma | 1 (2%) |
| Benign prostate hypertrophy | 1 (2%) |

Abbreviation: CCP, COVID-19 convalescent plasma.

Note: Data are represented as mean ± standard deviation or median (range) for continuous data and *n* (%) for categorical data.

CoV-2 antibody test results (data not shown). The results of the remaining 47 CCP donors (25 females, 22 males) were analyzed. Mean age (±SD) of CCP donors was 41.4 ± 13.7 years. Median duration after acute COVID-19 infection was 97 days (range, 34–401). Patient characteristics are presented in **Table 1**. None of the CCP donors developed a thrombotic event during or after COVID-19 infection until study inclusion. None of them was vaccinated against SARS-CoV-2 at the time of blood collection. In the control cohort, 51 (22 females, 29 males) plasma donors with a mean age of 38.9 ± 18.4 years, who did not have SARS-CoV-2 infection, were enrolled.

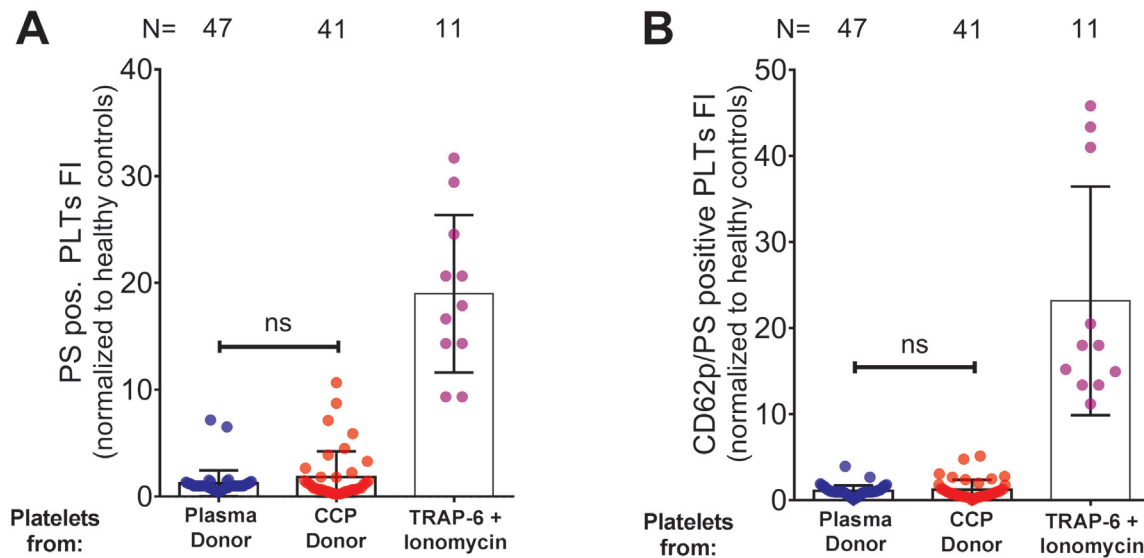


Fig. 1 Assessment of procoagulant platelet phenotype in COVID-19 convalescent plasma (CCP) donors. Procoagulant platelet phenotype was analyzed by assessing phosphatidylserine (PS) externalization (A) in platelets from plasma donors and CCP donors. Furthermore, procoagulant platelets were assessed (B) by using flow cytometry analysis of CD62p/PS double positive cells in platelets from healthy plasma and CCP donors (blue: plasma donors; red: CCP donors). Data are presented as mean \pm standard deviation of the fold increase (FI) of the mean fluorescence intensity (MFI) or percentage (%). ns, not significant.

No Increased Levels of Procoagulant Platelets in CCP Donors

We first measured PS externalization using annexin V. The rate of PS-positive cells was not different between plasma donors and CCP donors (FI of PS-positive platelets: 1.24 ± 1.21 [95% confidence interval [CI]: 0.89–1.6] vs. 1.83 ± 2.40 [95% CI: 1.07–2.6], respectively, $p = 0.43$; **Fig. 1A**). Similarly, the rate of double positive cells (CD62p and annexin V) was similar between plasma donors and CCP donors (FI of CD62p/PS-positive platelets: 1.09 ± 0.62 [95% CI: 0.91–1.27] vs. 1.22 ± 1.51 [95% CI: 0.85–1.58], respectively, $p = 0.29$; **Fig. 1B**). Baseline CD62p expressions (FI in mean fluorescence intensity [MFI]: 1.0 ± 0.13 [95% CI: 0.95–1.04] vs. 1.31 ± 1.20 [95% CI: 0.93–1.69], $p = 0.93$) were not statistically different between plasma donors and CCP donors. Moreover, CD62p release was comparable between both groups upon activation with TRAP-6 at $2.5 \mu\text{M}$ (FI of MFI: 1.59 ± 1.58 [95% CI: 1.1–2.08] vs. 1.69 ± 1.18 [95% CI: 1.28–2.01], $p = 0.43$) as well as with TRAP-6 at $10 \mu\text{M}$ (FI of MFI: 4.62 ± 4.42 [95% CI: 3.52–6.12] vs. 4.18 ± 3.28 [95% CI: 3.15–5.22], $p = 0.92$; **Fig. 2**). Furthermore, GPVI shedding at baseline (FI of GPVI-negative platelets: 1.0 ± 0.12 [95% CI: 0.96–1.04] vs. 1.31 ± 0.81 [95% CI: 1.06–1.55], $p = 0.08$) and after activation with $2.5 \mu\text{g/mL}$ CRP (FI of GPVI-negative platelets: 2.16 ± 1.16 [95% CI: 1.83–2.48] vs. 2.31 ± 1.21 [95% CI: 1.96–2.68], $p = 0.49$) were also similar between CCP donors and plasma donors (**Fig. 3**).

Antibody-Mediated Procoagulant Platelets and GPVI Using Washed Platelets

Compared with sera from noninfected plasma donors, sera from CCP donors did not induce higher PS externalization (FI of PS-positive platelets: 1.16 ± 0.66 [95% CI: 0.61–1.72] vs.

1.51 ± 0.74 [95% CI: 1.3–1.74], respectively, $p = 0.11$; **Fig. 4A**) or increased the rate of CD62p/PS double positive procoagulant phenotype (FI in CD62p/PS-positive platelets: 1.86 ± 0.87 [95% CI: 1.13–2.59] vs. 1.37 ± 0.63 [95% CI: 1.19–1.56], respectively, $p = 0.10$; **Fig. 4B**) in platelets from healthy persons. Of note, CD62p expression in healthy platelets after incubation with sera from CCP plasma donors was significantly lower compared with sera from noninfected donors (FI in CD62p: 2.09 ± 1.36 [95% CI: 0.95–3.24] vs. 1.16 ± 0.45 [95% CI: 1.03–1.30], $p < 0.01$; **Fig. 4C**). Sera-mediated GPVI shedding was similar between the groups (1.07 ± 0.16 [95% CI: 0.94–1.21] vs. 1.27 ± 0.91 [95% CI: 0.99–1.54], $p = 0.52$; **Fig. 4D**).

Thromboelastographic Assays

We assessed coagulation and fibrinolysis in whole blood samples using a thromboelastometry (**Table 2**, **Fig. 5**). The thromboelastometry was available in 39 CCP donors. We determined clotting time and maximum clot firmness in EX test as well as in FIB test to evaluate the coagulation. Only one CCP donor (case no. 7) had increased maximum clot firmness in EX test and FIB test. These parameters were *within the* normal range in other CCP donors. Second, we determined maximum clot lysis in EX test and in tPA test and lysis time in tPA test to evaluate the fibrinolysis. Again, one CCP donor (case no. 7) had increased lysis time in tPA test. This donor had arterial hypertension and diabetes mellitus type 2. Other CCP donors had normal fibrinolysis values.

Discussion

Recent studies have repeatedly shown that platelets are composed of different subpopulations that fulfill different

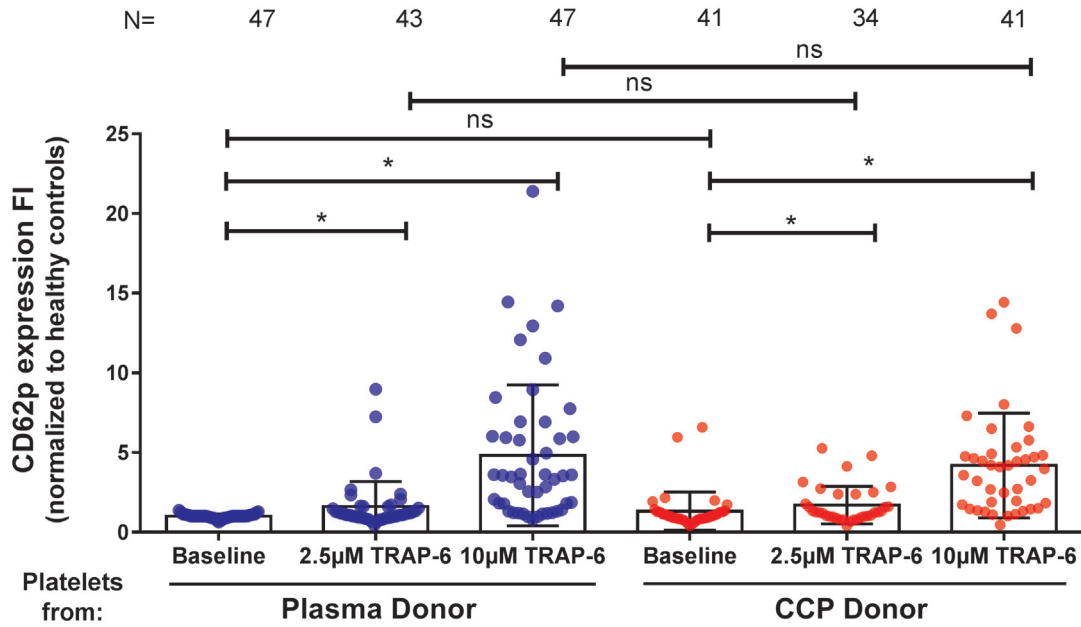


Fig. 2 CD62p expression on platelets from COVID-19 convalescent plasma (CCP) donors. The basal expression of P-selectin was determined on the surface of platelets from plasma and CCP donors. Where indicated, platelets were treated with thrombin receptor activating peptide (TRAP-6, 2.5 and 10 µM) before staining with anti-CD62p (blue: plasma donors; red: CCP donors). Data are presented as mean ± standard deviation of the fold increase (FI) of mean fluorescence intensity (MFI) or percentage (%). ns, not significant; **p* < 0.05.

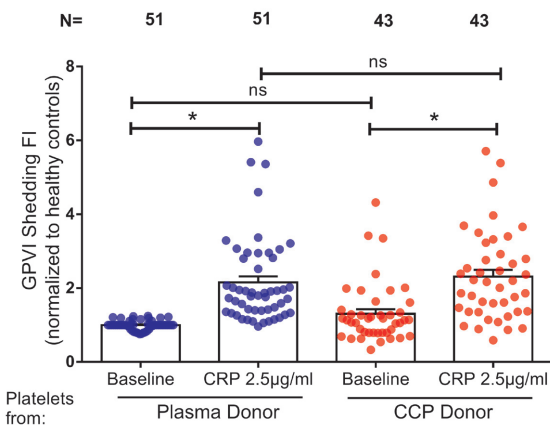


Fig. 3 GPVI shedding from platelet surface. Reduction in the surface expression of GPVI on the platelets of plasma and CCP donors was analyzed with or without incubation with collagen-related peptide (CRP, 2.5 µg/ml) by anti-GPVI-PE antibody staining (blue: plasma donors; red: CCP donors). Data are presented as mean ± standard deviation of the fold increase (FI) of percentage of GPVI-negative platelets (%). ns, not significant; **p* < 0.05.

roles in coagulation.¹⁹ Procoagulant platelets are a distinct subgroup that externalize PS on their surfaces and support fibrin formation.²⁰ Despite the vast amount of studies on coagulation in COVID-19, very few studies investigated procoagulant platelets during acute COVID-19 infection. Althaus et al have shown an increase in PS externalization in critically ill COVID-19 patients compared with noncritically ill COVID-19 patients.¹⁰ They also showed that PS externalization is associated with thrombosis and high SOFA scores in

this patient group.¹⁰ Interestingly, Denorme et al found a reduced PS externalization after dual agonist stimulation in COVID-19 patients compared with healthy donors.²¹ Similarly, Khattab et al demonstrated that procoagulant platelet levels are lower than controls in moderate and severe COVID-19 patients, but an increase in procoagulant platelets is associated with mortality in COVID-19 patients.¹¹ Previous studies reported on antibody-mediated increase in PS exposure as a marker for procoagulant platelets during severe COVID-19, via active engagement of FcγRIIA.^{9,10,22} However, this phenomenon seems to be limited to very severe COVID-19 patients and might be undetectable in small cohort of donors who had only mild SARS-CoV-2 infection. To our best knowledge, PS externalization of platelets in COVID-19 convalescent individuals has not been investigated before. In this study, we did not find a difference between CCP donors and controls in terms of PS externalization.

Surface expression of CD62p (P-selection) is a marker of platelet activation. Manne et al showed that CD62p expression is increased compared with controls in hospitalized COVID-19 patients.²³ Hottz et al found an increased CD62p expression in severe COVID-19 patients but not in patients with a mild or asymptomatic COVID-19 infection.⁸ Furthermore, CD62p surface expression at admission was correlated with D-dimer and associated with the need for mechanical ventilation as well as with in-hospital mortality, suggesting an association between platelet activation and COVID-19-associated coagulopathy.⁸ We found that CD62p expression in CCP donors at baseline and after stimulation with TRAP-6 was not higher compared with controls. These findings suggest that platelets of CCP donors are not activated.

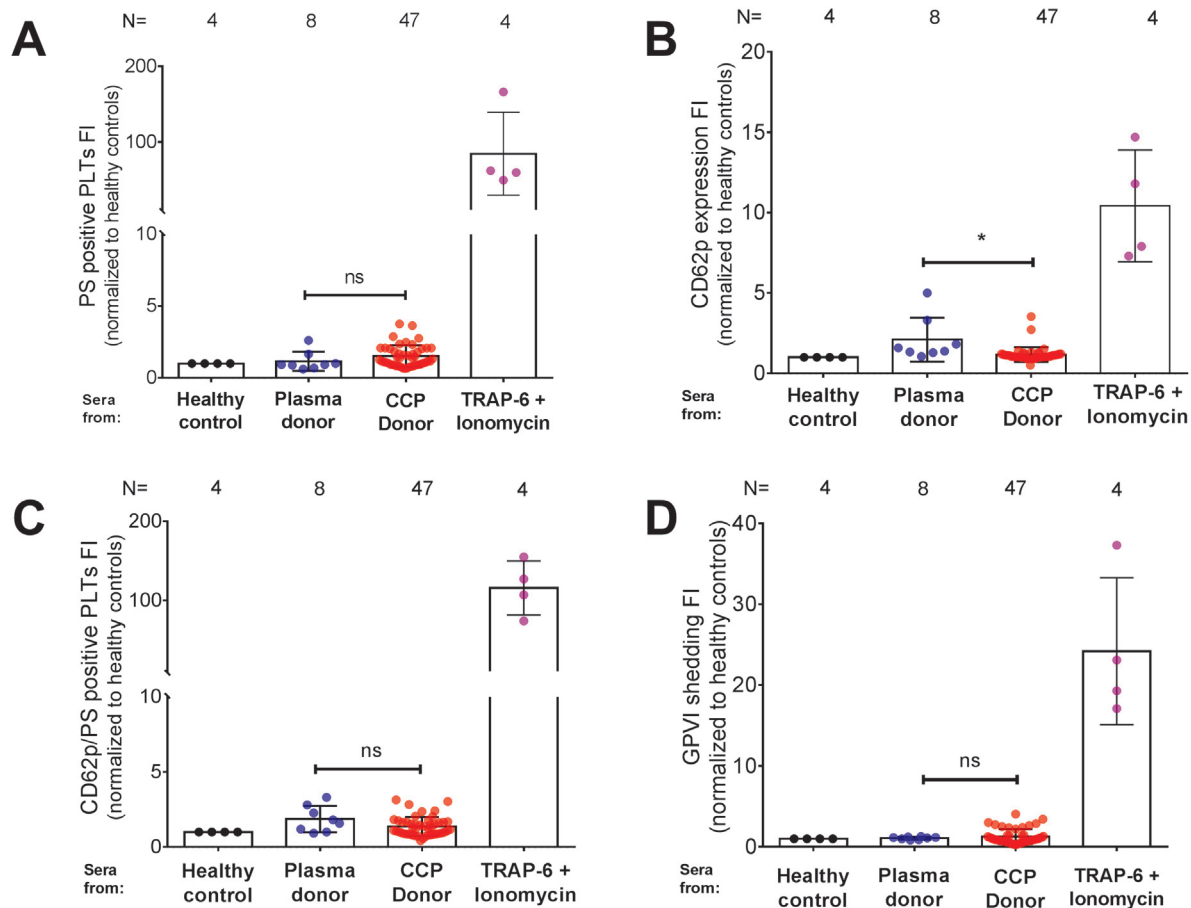


Fig. 4 Serum-induced effects on platelet's phenotype. Sera from healthy controls, plasma donors, and COVID-19 convalescent plasma (CCP) donors were incubated with washed platelets isolated from healthy donors, and PS externalization (A), procoagulant platelet formation (B), platelet activation (C), and GPVI shedding (D) were determined (blue: plasma donors; red: CCP donors). Each sample was tested with platelets from one donor. Data are presented as mean \pm standard deviation of the fold increase (FI) of healthy donors. ns, not significant; PS, phosphatidylserine; * $p < 0.05$.

Table 2 Viscoelastic properties of clots formed in blood samples from CCP donors

| Parameter | Test | Reference range | Mean | Standard deviation | Lower 95% CI of mean | Upper 95% CI of mean |
|----------------------------|----------|-----------------|------|--------------------|----------------------|----------------------|
| Clotting time (s) | EX test | 38–65 | 48 | 4.8 | 46 | 49 |
| Maximum clot firmness (mm) | EX test | 53–68 | 62 | 3.5 | 60 | 63 |
| Maximum lysis (%) | EX test | 0–12 | 5.6 | 2 | 4.9 | 6.2 |
| Maximum clot firmness (mm) | FIB test | 9–27 | 15 | 4.2 | 14 | 17 |
| Maximum lysis (%) | tPA test | 92–100 | 95 | 1.2 | 94 | 95 |
| Lysis time (s) | tPA test | <300 | 185 | 39 | 173 | 198 |

Abbreviations: CCP, COVID-19 convalescent plasma; CI, confidence interval.

Recent randomized trials showed increased survival in COVID-19 patients receiving CCP with high-dose neutralizing antibodies.^{17,18} However, the risk of exacerbation of COVID-19-associated coagulation derangements with CCP as well as transfusion-related complications have also been expressed.¹⁶ Therefore, investigation of the effect of CCP on platelets is of clinical importance. In this study, we showed

that sera from CCP donors do not induce a procoagulant phenotype or platelet activation in healthy platelets.

GPVI, the platelet immunoreceptor tyrosine-activating motif receptor for collagen, has been shown to play a prominent role on vascular integrity during inflammation.²⁴ Bongiovanni and colleagues reported that enhanced GPVI levels during SARS-CoV-2 infection might hint toward a

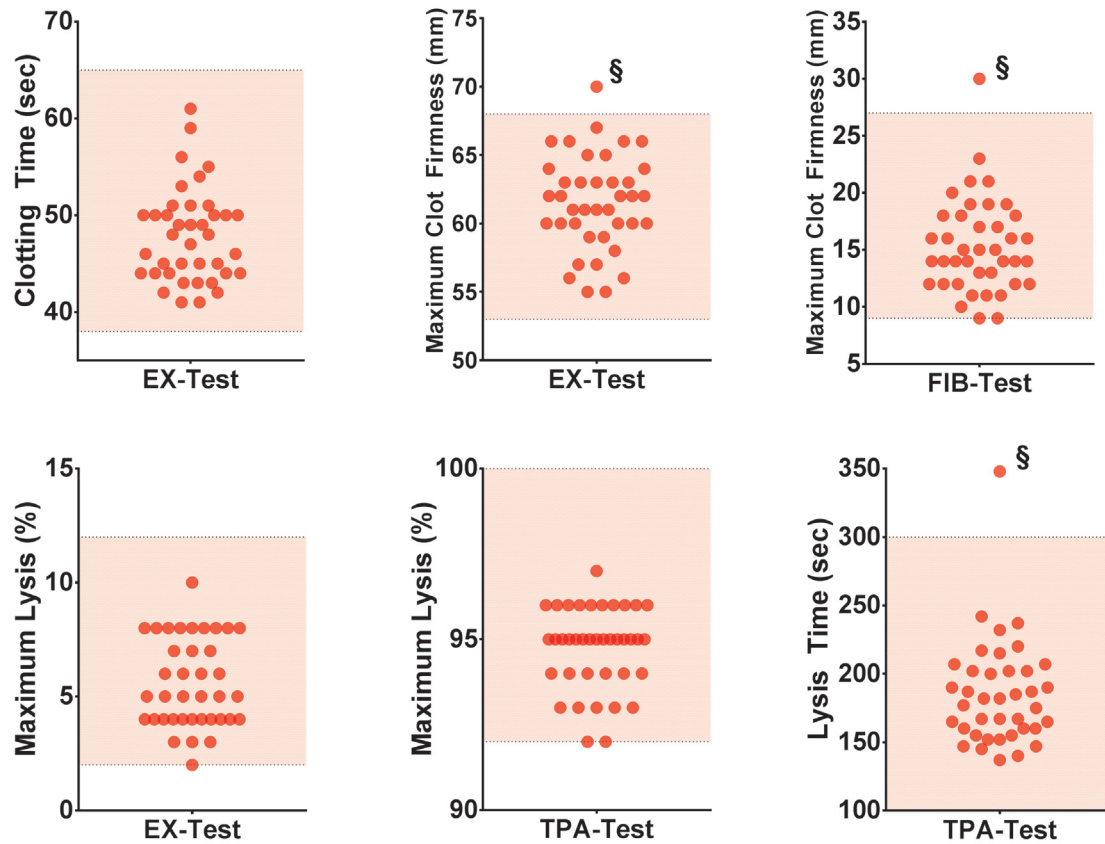


Fig. 5 Viscoelastic properties of clot formed in blood samples from COVID-19 convalescent plasma (CCP) donors. Citrated blood samples were collected and investigated within 2 hours using a viscoelastic test system (ClotPro Enicor GmbH, Munich, Germany). EX test, FIB test, and tPA test results in CCP donors. Pink area illustrates the reference ranges determined by the manufacturer; § denotes case no. 7.

hyperactivated phenotype of platelets during COVID-19, and this might play a role during hypercoagulopathy observed in COVID-19 and hence influence the patient outcome.²⁵ Apart from antibody-mediated procoagulant platelet generation, other mechanisms have also been reported previously. Costimulation of GPVI along with protease-activated receptors, PAR1 and PAR4, has been shown to increase PS exposure and subsequent procoagulant platelet formation.^{26,27} During dual stimulation of GPVI and PAR1/4, a sustained increase in Ca^{2+} levels leads to PS exposure in platelets.²⁸ Thus, it still remains unclear whether procoagulant platelet formation in severe COVID-19 is caused solely by antibody-mediated mechanisms or also by increased thrombin generation and higher levels of inflammation factors. In our study, no significant differences were observed in GPVI levels of CCP donors as compared with plasma donors at baseline as well as after stimulation with CRP. Similarly, sera from CCP donors as well as from plasma donors induced similar levels of GPVI cleavage from healthy platelets.

As more people recover from COVID-19, discussions have begun over the possibility of post-COVID syndrome or the so-called long-COVID syndrome.¹² A recent epidemiological study demonstrated a significantly increased readmission rate (3.5-fold, 95% CI: 3.4–3.6) and post-discharge mortality rate (7.7-fold, 95% CI: 7.2–8.3) in COVID-19 patients com-

pared with a non-COVID control group.²⁹ Several retrospective studies reported the rate of thromboembolic events after discharge in patients with COVID-19. The rates of venous thromboembolism and arterial thromboembolism in these studies are 0.2 to 2.6%^{30–35} and 0 to 1.9%,^{31–33} respectively. These studies are mostly retrospective and lack a comprehensive follow-up of the patients after discharge, which suggests that the true incidence of thromboembolic events could be even higher.

Very few studies investigated cellular and plasmatic components of the coagulation system after acute COVID-19 infection. Most recently, von Meijenfeldt and colleagues reported elevated plasma levels of factor VIII and PAI-1 in COVID-19 patients 4 months after discharge.¹³ Townsend et al found increased D-dimer values in both hospitalized and nonhospitalized patients at median of 80.5 days after initial diagnosis.¹⁴ In this study, we used thromboelastometry to evaluate the coagulation status in CCP donors. An increased maximum clot firmness and hypofibrinolysis in thromboelastometry have been reported in hospitalized COVID-19 patients. Hulshof et al reported an increased maximum clot firmness over 80% of all measurements in critically ill COVID-19 patients.³⁶ In the same study, a sufficient (>90%) clot breakdown was not achieved in more than half of the samples.³⁶ In a previous study from

our group, an increased maximum clot firmness and extended lysis time in COVID-19 patients admitted to normal wards or to the intensive care has been demonstrated.³⁷ The fibrin clots in the lungs of COVID-19 patients are more compact, consist of thin fibers, and have small pores compared with fibrin clots in patients with influenza infection.³⁸ Together with reduced fibrinolytic activity, this altered clot structure might cause thrombus in COVID-19 patients to be resistant to fibrinolysis. Two previous studies have investigated global coagulation status using rotational thromboelastometry after ICU discharge in COVID-19 patients.^{15,39} Magomedov et al reported that maximum clot firmness reduced significantly within 12 weeks after discharge in COVID-19 patients.³⁹ Most recently, Hulshof et al have shown that maximum clot firmness was within normal range in the tissue-type plasminogen activator rotational thromboelastometry in COVID-19 patients 6 months after discharge from ICU.¹⁵ However, although the lysis time in the same test overall significantly reduced 6 months after discharge, it remained over the normal range in 4 of 22 (18%) patients.¹⁵ Similarly, von Meijenfeldt reported a prolonged clot lysis time in COVID-19 patients 4 months after hospital discharge, suggesting a sustained hypofibrinolytic state.¹³ In this current study, we evaluated coagulation and fibrinolysis in CCP donors with rotational thromboelastometry. We demonstrated increased hypercoagulability and a hypofibrinolytic state in one donor (2%). This donor did not experience any thrombotic event during COVID-19 infection and thereafter. However, this donor had arterial hypertension and diabetes mellitus type 2. Yürekli et al found an increased maximum clot firmness in diabetic patients than in controls.⁴⁰ Comorbidities of this donor might be responsible for the abnormal findings in thromboelastometry. Further studies are needed to better define the risk of thrombosis after discharge and in the convalescent phase in COVID-19 patients. Of note, impaired fibrinolysis is not restricted to COVID-19 or sepsis. In an ongoing study, we observed an increased resistance to clot lysis in some patients with vascular occlusive disorder after stem cell transplantation (unpublished data). The clinical relevance of these findings has not been investigated yet.

Our study has several limitations. First, we did not have blood samples at the time of infection that would allow us to compare the changes in time. Second, this study is focused on platelets and we did not measure plasmatic coagulation factors in blood. Further studies should investigate the alterations in plasma components of coagulation and fibrinolytic system after acute COVID-19 infection. Finally, plasma donors undergo routine clinical examination as required by local regulations which may cause a selection bias since they are relatively younger and healthier compared with other COVID-19 convalescent individuals.

In conclusion, we could neither detect a procoagulant platelet phenotype or increased platelet activation nor a hypercoagulable or hypofibrinolytic state in CCP donors after primary infection. Moreover, sera from CCP donors

did not induce significant changes in platelet activation or procoagulant status. These findings support data from clinical studies which indicate that transfusion of CCP to treat or prevent severe COVID-19 is not associated with increased risk of exacerbation of the coagulopathy in COVID-19.

“What Is Known About This Topic?”

- Platelets contribute to the hypercoagulable state in COVID-19 patients.
- Platelets of critically ill COVID-19 patients express a procoagulant phenotype.
- Immunoglobulin G fractions from severe COVID-19 patients induce a procoagulant phenotype in healthy platelets.

“What Does This Paper Add?”

- Procoagulant platelet phenotypes were not observed after mild COVID-19 infection.
- Sera from CCP donors do not activate healthy platelets or induce procoagulant phenotype.

Author Contributions

G.U., A.S., L.P., K.A., P.B., H.K., and T.B. designed the study. G.U. and S.N.H. collected and analyzed the clinical data. G.U., A.S., W.A-K., L.P., K.W., and K.A. performed the experiments. G.U., A.S., P.B., H.K., and T.B. analyzed the data, interpreted the results, and wrote the manuscript. All authors read and approved the manuscript.

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Conflict of Interest

T.B. has received research funding from CoaChrom Diagnostica GmbH, DFG, Robert Bosch GmbH, Stiftung Transfusionsmedizin und Immunhämatologie e.V.: Ergomed, DRK Blutspendedienst, Deutsche Herzstiftung, Ministerium für Wissenschaft, Forschung und Kunst Baden-Wuerttemberg; has received lecture honoraria from Aspen Germany GmbH, Bayer Vital GmbH, Bristol-Myers Squibb GmbH & Co., Doctrina Med AG, Meet The Experts Academy UG, Schoechl Medical Education GmbH, Stago GmbH, Mitsubishi Tanabe Pharma GmbH, Novo Nordisk Pharma GmbH, Leo Pharma GmbH, Swedish Orphan Biovitrum GmbH; has provided consulting services to Terumo; has provided expert witness testimony relating to heparin-induced thrombocytopenia (HIT) and non-HIT thrombocytopenic and coagulopathic disorders. All of these are outside the current work. Other authors declare no competing financial interests.

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References

- Levi M, Thachil J. Coronavirus disease 2019 coagulopathy: disseminated intravascular coagulation and thrombotic microangiopathy—either, neither, or both. *Semin Thromb Hemost* 2020;46(07):781–784
- Bussani R, Schneider E, Zentilin L, et al. Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology. *EBioMedicine* 2020;61:103104
- Wichmann D, Sperhake J-P, Lütgehetmann M, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med* 2020;173(04):268–277
- Nadkarni GN, Lala A, Bagiella E, et al. Anticoagulation, bleeding, mortality, and pathology in hospitalized patients with COVID-19. *J Am Coll Cardiol* 2020;76(16):1815–1826
- Thachil J, Cushman M, Srivastava A. A proposal for staging COVID-19 coagulopathy. *Res Pract Thromb Haemost* 2020;4(05):731–736
- Iba T, Levy JH, Connors JM, Warkentin TE, Thachil J, Levi M. The unique characteristics of COVID-19 coagulopathy. *Crit Care* 2020;24(01):360
- Zaid Y, Puhm F, Allaes I, et al. Platelets can associate with SARS-CoV-2 RNA and are hyperactivated in COVID-19. *Circ Res* 2020;127(17):1404–1418
- Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood* 2020;136(11):1330–1341
- Zlamal J, Althaus K, Jaffal H, et al. Upregulation of cAMP prevents antibody-mediated thrombus formation in COVID-19. *Blood Adv* 2022;6(01):248–258
- Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. *Blood* 2021;137(08):1061–1071
- Khattab MH, Prodan CI, Vincent AS, et al. Increased procoagulant platelet levels are predictive of death in COVID-19. *Geroscience* 2021;43(04):2055–2065
- Balachandar V, Mahalaxmi I, Subramaniam M, et al. Follow-up studies in COVID-19 recovered patients - Is it mandatory? *Sci Total Environ* 2020;729:139021
- von Meijenfeldt FA, Havervall S, Adelmeijer J, et al. Sustained prothrombotic changes in COVID-19 patients 4 months after hospital discharge. *Blood Adv* 2021;5(03):756–759
- Townsend L, Fogarty H, Dyer A, et al. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. *J Thromb Haemost* 2021;19(04):1064–1070
- Hulshof A-M, Braeken DCW, Ghossein-Doha C, et al. Hemostasis and fibrinolysis in COVID-19 survivors 6 months after intensive care unit discharge. *Res Pract Thromb Haemost* 2021;5(06):e12579
- Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19 convalescent plasma in 5000 patients. *J Clin Invest* 2020;130(09):4791–4797
- Körper S, Weiss M, Zickler D, et al; CAPSID Clinical Trial Group. Results of the CAPSID randomized trial for high-dose convalescent plasma in patients with severe COVID-19. *J Clin Invest* 2021;131(20):e152264
- O'Donnell MR, Grinsztejn B, Cummings MJ, et al. A randomized double-blind controlled trial of convalescent plasma in adults with severe COVID-19. *J Clin Invest* 2021;131(13):150646
- Heemskerk JWM, Mattheij NJA, Cosemans JMEM. Platelet-based coagulation: different populations, different functions. *J Thromb Haemost* 2013;11(01):2–16
- Tohidi-Esfahani I, Lee CSM, Liang HPH, Chen VMY. Procoagulant platelets: Laboratory detection and clinical significance. *Int J Lab Hematol* 2020;42(Suppl 1):59–67
- Denorme F, Manne BK, Portier I, et al. COVID-19 patients exhibit reduced procoagulant platelet responses. *J Thromb Haemost* 2020;18(11):3067–3073
- Pelzl L, Singh A, Funk J, et al. Antibody-mediated procoagulant platelet formation in COVID-19 is AKT dependent. *J Thromb Haemost* 2022;20(02):387–398
- Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood* 2020;136(11):1317–1329
- Weiss LJ, Manukjan G, Pflug A, et al. Acquired platelet GPVI receptor dysfunction in critically ill patients with sepsis. *Blood* 2021;137(22):3105–3115
- Bongiovanni D, Klug M, Lazareva O, et al. SARS-CoV-2 infection is associated with a pro-thrombotic platelet phenotype. *Cell Death Dis* 2021;12(01):50
- Alberio L, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of alpha-granule factor V in human platelets: effects of ionophore A23187, thrombin, collagen, and convulxin. *Blood* 2000;95(05):1694–1702
- Reddy EC, Rand ML. Procoagulant phosphatidylserine-exposing platelets *in vitro* and *in vivo*. *Front Cardiovasc Med* 2020;7:15
- Harper MT, Londoño JEC, Quick K, et al. Transient receptor potential channels function as a coincidence signal detector mediating phosphatidylserine exposure. *Sci Signal* 2013;6(281):ra50
- Ayoubkhani D, Khunti K, Nafilyan V, et al. Post-COVID syndrome in individuals admitted to hospital with COVID-19: retrospective cohort study. *BMJ* 2021;372(693):n693
- Rashidi F, Barco S, Kamangar F, et al. Incidence of symptomatic venous thromboembolism following hospitalization for coronavirus disease 2019: Prospective results from a multi-center study. *Thromb Res* 2021;198:135–138
- Salisbury R, Iotchkova V, Jaafar S, et al. Incidence of symptomatic, image-confirmed venous thromboembolism following hospitalization for COVID-19 with 90-day follow-up. *Blood Adv* 2020;4(24):6230–6239
- Bourguignon A, Beaulieu C, Belkaid W, Desilets A, Blais N. Incidence of thrombotic outcomes for patients hospitalized and discharged after COVID-19 infection. *Thromb Res* 2020;196:491–493
- Patell R, Bogue T, Koshy A, et al. Postdischarge thrombosis and hemorrhage in patients with COVID-19. *Blood* 2020;136(11):1342–1346
- Roberts LN, Whyte MB, Georgiou L, et al. Postdischarge venous thromboembolism following hospital admission with COVID-19. *Blood* 2020;136(11):1347–1350
- Engelen MM, Vanassche T, Balthazar T, et al. Incidence of venous thromboembolism in patients discharged after COVID-19 hospitalisation. *Res Pract Thromb Haemost* 2020;4(Suppl 1)<https://abstracts.isth.org/abstract/incidence-of-venous-thromboembolism-in-patients-discharged-after-covid-19-hospitalisation/>
- Hulshof A-M, Brüggemann RAG, Mulder MMG, et al. Serial EXTEM, FIBTEM, and tPA rotational thromboelastometry observations in the Maastricht intensive care COVID Cohort - persistence of hypercoagulability and hypofibrinolysis despite anticoagulation. *Front Cardiovasc Med* 2021;8:654174
- Hammer S, Häberle H, Schlensak C, et al. Severe SARS-CoV-2 infection inhibits fibrinolysis leading to changes in viscoelastic properties of blood clot: a descriptive study of fibrinolysis in COVID-19. *Thromb Haemost* 2021;121(11):1417–1426

- 38 Wygrecka M, Birnhuber A, Seeliger B, et al. Altered fibrin clot structure and dysregulated fibrinolysis contribute to thrombosis risk in severe COVID-19. *Blood Adv* 2022;6(03): 1074–1087
- 39 Magomedov A, Zickler D, Karaivanov S, et al. Viscoelastic testing reveals normalization of the coagulation profile 12 weeks after severe COVID-19. *Sci Rep* 2021;11(01): 13325
- 40 Yürekli BPS, Ozcebe OI, Kirazli S, Gürlek A. Global assessment of the coagulation status in type 2 diabetes mellitus using rotation thromboelastography. *Blood Coagul Fibrinolysis* 2006;17(07): 545–549