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ORIGINAL ARTICLE

Prophylactic fresh frozen plasma and platelet transfusion have a prothrombotic effect in patients with liver disease

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Abstract

Background and Aims: Patients with liver disease acquire complex changes in their hemostatic system, resulting in prolongation of the international normalized ratio and thrombocytopenia. Abnormalities in these tests are commonly corrected with fresh frozen plasma (FFP) or platelet transfusions before invasive procedures. Whether these prophylactic transfusions are beneficial and truly indicated is increasingly debated. In this study, we studied ex vivo effects of FFP and platelet transfusions in patients with liver disease-associated hemostatic changes in a real-life clinical setting.

Methods: We included 19 patients who were deemed to require prophylactic FFP transfusion by their treating physician and 13 that were prescribed platelet transfusion before a procedure. Hemostatic status was assessed in blood samples taken before and after transfusion and compared with healthy controls (n = 20).

Results: Ex vivo thrombin generation was preserved in patients with liver disease before FFP transfusion. Following FFP transfusion, both in and ex vivo thrombin generation significantly increased, as evidenced by a 92% and 38% increase in thrombin-antithrombin and prothrombin fragment 1 + 2 levels, respectively, and a 20% increase in endogenous thrombin potential. Platelet counts increased from 28 [21-41] $\times 10^9$/L before to 43 [39-64] $\times 10^9$/L after platelet transfusion ($P < .01$), and was accompanied by increases in in vivo markers of hemostatic activation.

Conclusions: FFP and platelet transfusion resulted in increased thrombin generation and platelet counts in patients with liver disease, indicating a prothrombotic effect. However, whether all transfusions were truly indicated and had a clinically relevant effect is questionable.

KEYWORDS

cirrhosis, coagulation, fresh frozen plasma, neutrophil extracellular traps, platelets, transfusion

F.A.v.M. and B.P.v.d.B. are joint first authors; T.L. and W.B. are joint senior authors.

1 | INTRODUCTION

Patients with liver disease frequently acquire complex alterations in their hemostatic system. Their conventional coagulation tests often show a prolonged international normalized ratio (INR) and activated partial thromboplastin time (APTT), and/or low platelet counts, that historically were interpreted as implying an increased bleeding tendency. It is common clinical practice to correct these tests with fresh frozen plasma (FFP) or platelet transfusions prior to invasive procedures.[1,2] However, whether the prophylactic use of FFP and platelets in these patients is beneficial and truly indicated is increasingly debated.[3] This debate started after clinical and laboratory studies demonstrated patients with liver disease are not overtly coagulopathic, but rather characterized by a rebalanced hemostatic system, in which simultaneous changes in the pro- and antihemostatic factors result in a reset of the hemostatic balance. This balance, however, is less stable than the balance in people with intact liver function, and can be relatively easy tipped over to bleeding or thrombosis.[4]

Bleeding is a particularly feared complication in patients with liver disease. Clinicians have long sought guidance on identifying which patients are at increased risk for bleeding complications and might need prophylactic blood product transfusion. However, bleeding is not necessarily related to failing hemostasis, but often related to portal hypertension or mechanical vascular injury,[5,6] and standard diagnostic tests of hemostasis are poor predictors of bleeding risk.[7] Most recent guidelines therefore no longer recommend the use of conventional coagulation tests, such as the INR, in the assessment of bleeding risk in patients with liver disease.[8-10] The INR does not accurately reflect the hemostatic status of these patients because it is sensitive for procoagulant factors, but not for anticoagulant factors (which generally decrease to a similar extent). As a consequence, the INR overestimates the bleeding tendency in patients with liver disease. Despite the recommendations not to correct the INR with fresh frozen plasma,[8-10] threshold values for conventional coagulation tests (INR > 1.5) are often maintained by proceduralists in clinical practice, resulting in the frequent use of FFP transfusions before procedures.[1,11]

Although there is increasingly broad consensus on the futility of INR correction with FFP for preventing bleeding complications in this setting, there is none on the requirement to correct cirrhotic thrombocytopenia. High-quality research in this specific field is scarce, and clinical studies have shown contradicting results.[12,13] Guidelines defining the indication for platelet transfusion in patients with liver disease-associated thrombocytopenia are not uniform, although most guidelines maintain a platelet count threshold of 50×10^9/L for patients with liver disease before invasive procedures.[14]

In addition to doubts on whether prolonged INR and thrombocytopenia in cirrhosis are associated with bleeding risk, the efficacy of FFP and platelet transfusions in patients with liver disease has been questioned. In vitro and in vivo studies have shown that although FFP improves the INR in patients with liver disease, there is no appreciable increase in hemostatic potential as estimated by thrombin generation tests.[15-17] In addition, several clinical studies have shown that FFP or platelet transfusions only minimally improve the hemostatic status of patients with liver disease and did not reduce bleeding risk.[17-19]

Essentials

- Abnormalities in standard coagulation tests in patients with liver disease are commonly corrected with blood product transfusions prior to invasive procedures. Whether these transfusions are truly indicated is increasingly debated.
- In this observational cohort study, we assessed the hemostatic status of patients with liver disease before and after transfusions.
- Blood product transfusion had a prothrombotic effect, as evidenced by increased thrombin generation, in vivo activation of coagulation and platelet counts following transfusion.
- Our findings contribute to a more rational approach to blood product transfusion practice in patients with liver disease.

Importantly, bleeding risk is, next to a patient's hemostatic status, determined by the procedure and operator. Extensive research has shown that many of the common procedures that patients with liver disease undergo (eg, endoscopy, paracentesis), have a low to very low bleeding risk, which further questions the need for prophylactic prohemostatic interventions.[6] Further, every transfusion is associated with the risk of transfusion-related complications, such as circulatory overload, transfusion-related acute lung injury, and infection, and those risks may be higher in patients with liver disease.[20-22]

Here, we examined the hemostatic efficacy of prophylactic FFP and platelet transfusions to patients with chronic liver disease-associated hemostatic changes. We specifically studied patients that were deemed to require FFP or platelet transfusion before an invasive procedure by their treating physician in a real-life clinical setting.

2 | MATERIALS AND METHODS

2.1 | Study population

We studied patients with a prolonged INR and/or thrombocytopenia related to chronic liver disease who were administered prophylactic FFP and/or platelet transfusions before an invasive procedure, as determined by their direct clinical care team. Patients were recruited between June 2019 and December 2019 at King's College Hospital (KCH) London. Ethical approval from the Health Research Authority and Health Care and Research Wales, Study Number 19/WA/0168, was obtained. The study protocol was approved by the Health Research Authority and Health Care and Research Wales,

and the Research and Innovation department at KCH; good clinical practice guidelines were followed. All patients, or in the case of incapacity, their next of kin, gave informed written consent/assent for participation in this study. Patients that were actively bleeding were excluded from this study. The other exclusion criteria were disseminated malignancy, hereditary thrombophilia or hemophilia, HIV positivity, pregnancy, or age younger than 18 years. Twenty healthy volunteers were recruited at KCH to establish reference values for the various laboratory tests performed. Exclusion criteria for healthy volunteers were similar to those applied in patients, with the addition of use of anticoagulant medications, history of venous thromboembolic events, and blood (product) transfusion up to 7 days before inclusion.

2.2 | Study procedures

Blood samples were collected before and after blood product transfusion. Blood for hemostasis studies was collected via venipuncture or from arterial or venous lines into vacuum tubes (Greiner Bio-One, Alphen a/d Rijn, the Netherlands) containing 3.2% trisodium citrate at a blood to anticoagulant ratio of 9:1, and vacuum EDTA tubes for determination of a complete blood cell count. Sodium, potassium, creatinine, albumin, total bilirubin, aspartate transaminase, gamma-glutamyltransferase, and alkaline phosphatase were measured as part of routine clinical care by the Blood Sciences Laboratory at KCH. Blood collected in citrate tubes was processed to platelet-poor plasma immediately following procurement of the sample by double centrifugation at 2000*g* and 10,000*g* for 10 minutes at 18°C. Plasma was stored at −80°C until used for analyses.

2.3 | Assessment of patient's hemostatic status in plasma

To study the effect of FFP transfusion on the hemostatic status of patients with liver disease, we performed thrombomodulin-modified thrombin generation assays in platelet-poor plasma using the fluorimetric method as described by Hemker et al.[23] We used commercially available reagents (FluCa-kit TS50.00, Thrombin Calibrator TS20.00, PPP reagent; Thrombinoscope, Maastricht, The Netherlands) containing recombinant tissue factor (final concentration, 5 pM), and phospholipids (final concentration, 4 μM) and soluble thrombomodulin (TS70.00, concentration not disclosed by manufacturer). Experiments were performed following protocols provided by the manufacturer.

Additional conventional coagulation tests, more specifically APTT, INR, factor II, and antithrombin activity, were performed using the StaCompact 3 (Stago, Breda, the Netherlands) using reagents and instructions from the manufacturer. Fibrinogen levels in plasma were determined using a Clauss fibrinogen assay on the ACL top 300 from Instrumentation laboratories (Werfen, Breda, the Netherlands) using the manufacturer's reagents and instructions.

2.4 | Assessment of activation of coagulation, inflammation, and cell-free DNA

We measured levels of thrombin/anti thrombin complexes (TAT) and prothrombin fragment 1 + 2 (F1 + 2), which are markers for in vivo thrombin generation, using the Enzygnost TAT micro and Enzygnost F1 + 2 ELISA kit (Siemens Healthcare Diagnostics, the Hague, the Netherlands), respectively. In vivo platelet activation was assessed by measuring levels of soluble CD40 ligand in plasma using a commercially available ELISA (DuoSet, R&D Systems, Bio-techne, UK). Interleukin-6 (IL-6) and C-reactive protein (CRP) levels were determined with the use of the Human IL-6 and Human CRP Quantikine ELISA kit (R&D Systems, Bio-techne). All tests were carried out according to the manufacturers' protocol. Levels of cell-free DNA and myeloperoxidase-DNA (MPO-DNA) complexes, were measured to assess neutrophil extracellular trap (NET) formation in plasma. Cell-free DNA was quantified using the Quant-iT PicoGreen dsDNA assay kit (Fisher Scientific, Landsmeer, the Netherlands) as described previously.[24] The concentration of MPO-DNA complexes in plasma was determined by ELISA, as previously described.[25]

2.5 | Statistical analyses

Statistical analyses were performed using GraphPad Prism version 8.3.1 (San Diego, CA). The results were presented as numbers and percentages for categorical variables and medians ± interquartile ranges for continuous variables. Plasma levels of the various markers of interest in liver patients before transfusion were compared with plasma levels after transfusion with the use of the Wilcoxon test for paired data. Comparisons with healthy controls were made using the Mann-Whitney *U* test. *P* values < .05 were considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics and procedural variables

From June 2019 to December 2019, a total of 19 patients with chronic liver disease and a prolonged INR that received prophylactic FFP transfusion before an invasive procedure were included in this study. In that same period, 13 patients with thrombocytopenia that received prophylactic platelet transfusion were included in this study. The decision to administer FFP or platelets was made by the treating clinical team. Patient characteristics and details of procedures and blood products transfused are summarized in Table 1. All patients underwent low- to intermediate-risk procedures, as defined by expert consensus.[26] In the FFP group, 53% of the patients underwent low-risk procedures and 47% underwent intermediate-risk procedures, of which all except for one were transjugular liver biopsies. Of the patients receiving platelet transfusion, 62% and 38% of the patients underwent

TABLE 1 Patient characteristics, procedural and blood product transfusion variables

Variable	Patients Receiving FFP[a] (n = 19)	Patients Receiving Platelets[a] (n = 13)	Healthy Controls (n = 20)
Age	50 [44-54]	48 [43-58]	44 [39-50]
Female	10 (53%)	7 (54%)	9 (45%)
Body mass index (kg/m^2)	24 [23-28]	27 [23-32]	24 [20-27]
Cardiovascular disease (yes)	7 (37%)	5 (38%)	0
Diabetes (yes)	3 (16%)	4 (31%)	1 (5%)
Infection (yes)	5 (26%)	6 (46%)	0
Sepsis (yes)	4 (21%)	4 (31%)	
Etiology of liver disease			
ALD	12[b,c]	5	
Autoimmune	4	0	
Cryptogenic	2	1	
NAFLD	0	4[d]	
Other[e]	0	2	
Viral	1[c]	1	
Ascites (no: slight: moderate: severe)	3: 5: 1: 10	0: 4: 7: 2	
Hepatic encephalopathy (yes)	4 (21%)	5 (38%)	
ACLF CLIF-C ACLF score	4 (21%) 70 [67-73] (n = 4)	6 (46%) 65 [63-66] (n = 6)	
Renal replacement therapy	4 (21%)	6 (46%)	
Mechanical ventilation	4 (21%)	4 (31%)	
Use of vasopressors	3 (16%)	4 (31%)	
Ward: intensive care unit	14:5	7:6	
Baseline laboratory values			
MELD score	25 [21-37]	21 [17-38]	
Child-Pugh (A: B: C)	0: 6: 13	2: 3: 8	
CLIF-C AD score	54 [44-64] (n = 15)	42 [35-46] (n = 7)	
Albumin (g/L)	30 [27-33]	30 [28-33]	
Alkaline phosphatase (IU/L)	150 [130-205]	116 [84-166]	
Aspartate aminotransferase (IU/L)	133 [69-205]	53 [39-67]	
Creatinine (μmol/L)	80 [61-116]	101 [90-130]	
Fibrinogen (g/L)	1.8 [1.5-2.8]	1.8 [1.3-2.6]	3.0 [2.8-3.5]
Gamma-glutamyltransferase (IU/L)	96 [72-181]	85 [46-133]	
INR	2.0 [1.8-2.4]	1.5 [1.4-2.3]	1.0 [1.0-1.1]
Platelet count (× 10^9/L)	76 [56-160]	28 [21-41]	244 [188-302]
Potassium	4.1 [4.0-4.7]	4.4 [3.9-4.8]	
Sodium (mmol/L)	136 [135-137]	138 [136-140]	
Total bilirubin (μmol/L)	249 [62-383]	136 [22-244]	
Transfusion variables			
Volume (mL); Volume (mL/kg)	835 [547-1070][f]; 10.7 [8.5-13.1]	302 [287-306]	
Units	4 [2-4]	1[g]	
Indication for transfusion			
Bronchoscopy	0	1	
Endoscopy (OGD)[h]	6	2	
Interventional Radiology[i]	1	2	

(Continues)

TABLE 1 (Continued)

Variable	Patients Receiving FFP[a] (n = 19)	Patients Receiving Platelets[a] (n = 13)	Healthy Controls (n = 20)
Line change	0	1	
Low platelet count	0	2	
Paracentesis	3	1	
Tracheostomy	1	1	
(Transjugular) liver biopsy	8	3	
Blood sampling (second sample)			
Before procedure	10 (53%)	10 (77%)	
During procedure	3 (16%)	0 (0%)	
After procedure	6 (32%)	3 (23%)	
Time between sampling and infusion last bag (min)	14 [5-47]	27 [9-33]	

Note: The results are presented as median [interquartile range] for continuous variables, and number (percentage) for categorical variables. Comparisons between the three groups were made using the one-way ANOVA test.

Abbreviations: ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; FFP, fresh frozen plasma; HCC, hepatocellular carcinoma; INR, international normalized ratio; MELD, model for end-stage liver disease; NAFLD, nonalcoholic fatty liver disease; OGD, esophago-gastro-duodenoscopy; TIPS, transjugular intrahepatic porto-systemic shunt.

[a]One patient received 4 units of Octaplas (solvent/detergent treated plasma) with a total volume of 800 mL instead of FFP. Four of the 13 patients received apheresed platelets instead of pooled platelets.

[b]One patient had a combined etiology of ALD + hepatitis C.

[c]Two patients were diagnosed with HCC on the background of cirrhosis (one with an etiology of ALD, one with hepatitis B).

[d]Three patients had combined etiologies with NAFLD; specifically, biopsy proven ALD/NAFLD, alpha-1 antitrypsin deficiency, and IgG4 multisystem disease.

[e]Other etiologies included recurrent graft cirrhosis of unknown cause, and Gilbert's syndrome + portal vein thrombosis.

[f]As per local protocols, FFP was transfused aiming to achieve 15 mL/kg (10-15 mL/kg as per international transfusion guidelines[8,9]). Three patients that received FFP transfusion also received platelet concentrate (volumes 227, 453, 303 mL) prior to the procedure and our second blood sample.

[g]One patient received 2 units of platelet concentrate with a total volume of 576 mL.

[h]All except one patient underwent esophago-gastro-duodenoscopy with therapeutic intent for treatment of oesophageal varices.

[i]The patient receiving FFP transfusion underwent TIPSogram. Procedures in the platelet group were splenic artery embolization, and TIPS.

low- and intermediate-risk procedures, respectively. We took blood samples before and after blood product transfusion and aimed to take the second blood sample before initiation of the procedure. However, because this was a clinical observational study in a real-life setting, in which we did not intervene with clinical care, 9/19 and 3/13 of the second blood samples in the FFP and platelet concentrate group, respectively, were taken during or after the procedure.

3.2 | Conventional coagulation tests are improved after FFP transfusion

Conventional coagulation tests were performed to determine the hemostatic status of liver patients before and after FFP transfusion (Figure 1). The INR significantly decreased by 18 [14-22]% after FFP transfusion from 1.97 [1.79-2.41] before to 1.68 [1.51-1.77] after transfusion. Similarly, the APTT decreased by 14 [9-21]% from 44.8 [38.7-52.1] seconds before to 37.4 [34.7-43.6] seconds after FFP transfusion. Factor II, AT, and fibrinogen levels significantly increased after FFP transfusion by 32%, 50%, and 14%, respectively, but remained significantly different from healthy controls.

3.3 | Thrombin generation is enhanced after FFP transfusion

Thrombin generation parameters before and after FFP transfusion are shown in Figure 2. At baseline, thrombomodulin-modified thrombin generation did not differ significantly between patients and controls. Total thrombin generation as assessed by the endogenous thrombin potential (ETP) increased by 20 [−3 to 40]% after FFP transfusion from 571 [448-659] nmol/L IIa × minutes before transfusion to 702 [591-787] nmol/L IIa × minutes after transfusion. The ETP after FFP transfusion was significantly higher than the ETP in healthy controls (702 vs 495 nmol/L IIa × minutes, $P < .01$). Not all patients had an increase in ETP after FFP transfusion. Seven (37%) showed either minimal increase or decrease in ETP: five patients (26%) had an ETP decrease between 5% and 30%, and two (11%) patients had a change in ETP of <5%. Of the 12 patients that showed an increase in ETP after FFP transfusion, eight increased by 10% to 50% and four had an increase of >50%. The peak thrombin increased from 83 nmol/L IIa (58-100) to 111 nmol/L IIa [81-127] after FFP transfusion, corresponding with a 30 [16-51]% increase, and peak thrombin after FFP transfusion was higher compared with levels in healthy controls, although this did not reach statistical

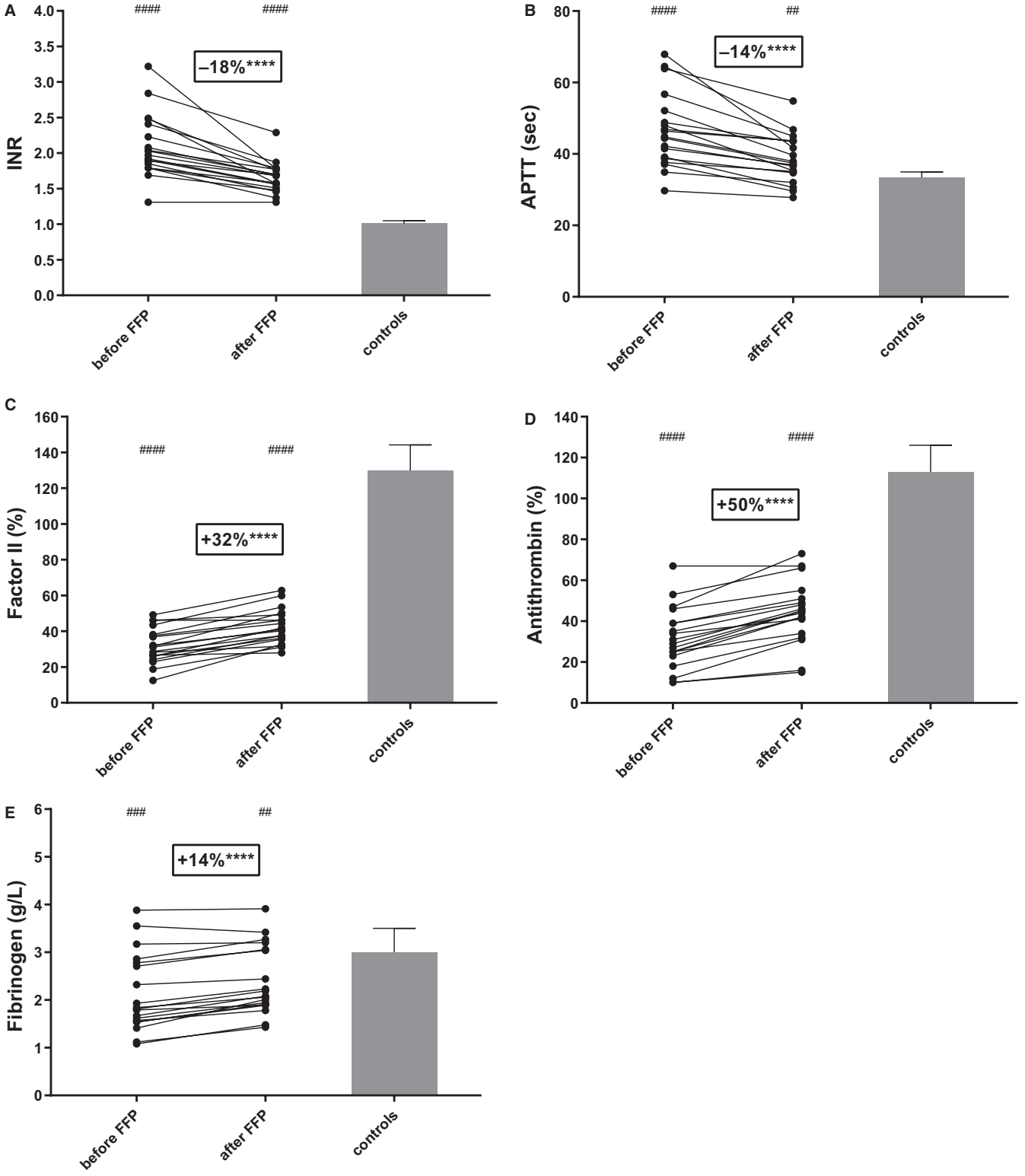

FIGURE 1 Conventional coagulation tests in patients with a prolonged INR and liver disease (n = 19) before and shortly after FFP transfusion. Patient tests were compared with tests in healthy controls (n = 20). Patient tests are depicted as individual dots connected by a line from before to after transfusion. Bars represent median ± interquartile ranges. $^{****}P < .0001$ before vs after FFP transfusion, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ patients vs controls. APTT, activated partial thromboplastin time; FFP, fresh frozen plasma; INR, international normalized ratio

significance (111 vs 81 nmol/L IIa, $P = .42$). Of the 19 patients, two had a decrease in peak thrombin between 5% and 30%, one did not change (<5%), 11 increased by 10% to 50%, and five increased by >50%. The lag time decreased in plasma taken before and after FFP transfusion, but was similar between patients and controls both before and after transfusion. In addition, the velocity index increased significantly by 24

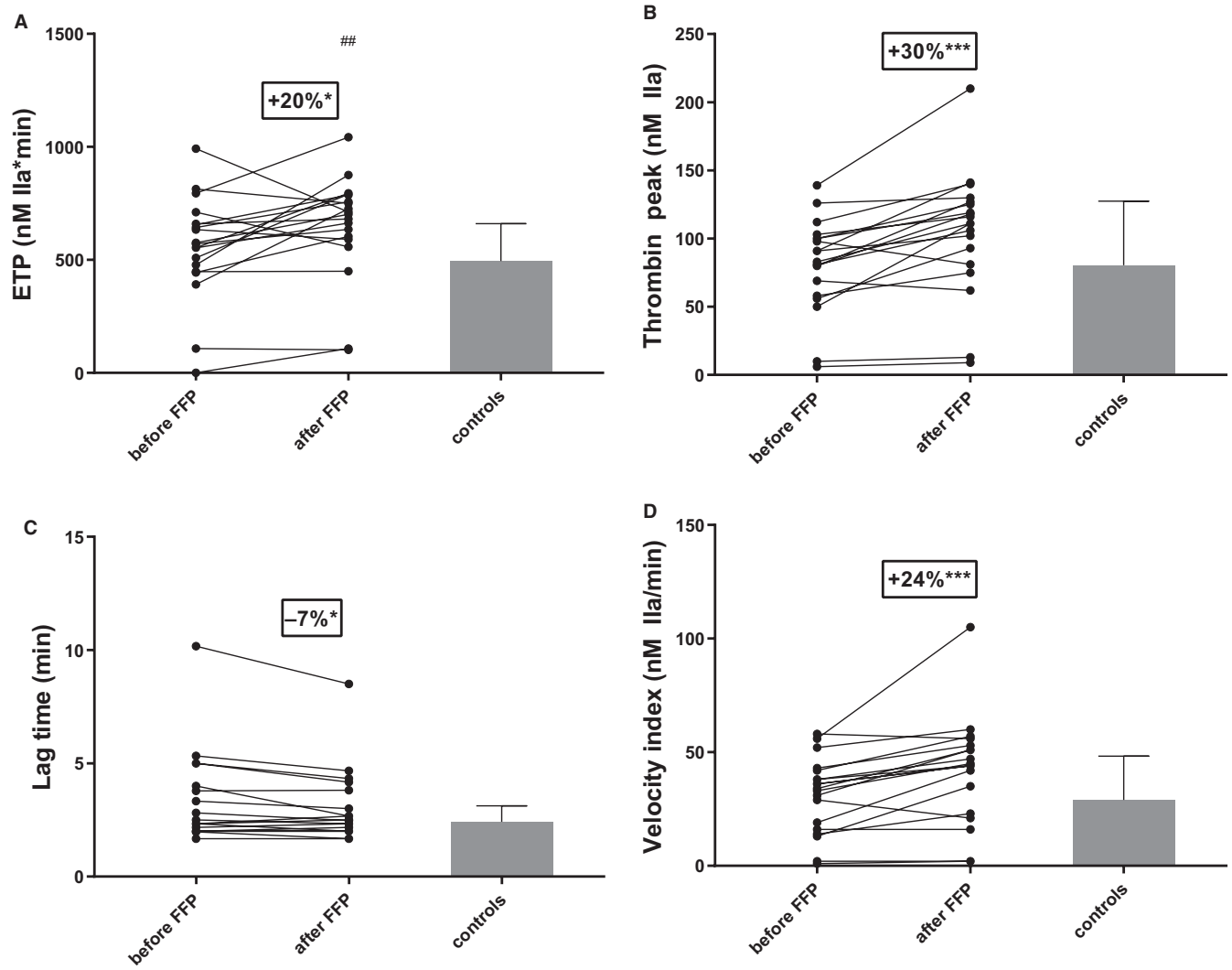

FIGURE 2 Parameters of thrombomodulin-modified thrombin generation assays in patients with a prolonged INR and liver disease (n = 19) before and after FFP transfusion. Parameters were compared with healthy controls (n = 20). Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. $^{*}P < .05$, $^{***}P < .001$, before vs after FFP transfusion, $^{\#\#}P < .01$ patients vs controls. ETP, endogenous thrombin potential; FFP, fresh frozen plasma; INR, international normalized ratio

[16-64]% after transfusion, and was higher in comparison to healthy controls (44 [23-53] vs 29 [19-48] nmol/L IIa/min, $P = .24$). Two patients had a remarkably low ETP, peak thrombin, and velocity index, as depicted in Figure 2A,B,D. These patients received low molecular weight heparin 24 to 36 hours before inclusion in this study, and had detectable anti-Xa activity at the time of blood sampling.

3.4 | In vivo *activation of coagulation following FFP transfusion*

Markers for activation of coagulation significantly increased after FFP transfusion, as depicted in Figure 3. Levels of TAT increased from 5.0 [4.0-9.6] μg/mL before to 11.8 [8.4-23.8] μg/mL after FFP transfusion, and levels of F1 + 2 increased from 224 [144-366] pmol/L to 326 [191-451] pmol/L after transfusion. At baseline, levels of TAT, but not F1 + 2, were significantly higher than levels found in healthy controls. After FFP transfusion, both TAT and F1 + 2 levels were higher in patients in comparison to healthy controls. The increase in TAT and F1 + 2 after transfusion of FFP, was substantial in some and virtually absent in other patients. There was no relation between the increase in TAT or F1 + 2 and severity of disease, or with timing of the second blood sample (before or during/after procedure).

3.5 | Mixed inflammatory responses following FFP transfusion

The inflammatory response to FFP transfusion was assessed by measuring IL-6 and CRP levels before and after FFP transfusion (Figure 4A,B). At baseline, the plasma level of IL-6 was significantly higher in patients than in controls. After transfusion, IL-6 level increased by 25 [−2 to 63]% from 37.1 [9.6-378] pg/mL to 50.6 [22.2-615] pg/mL, $P = .055$. Of the 19 patients, only 13 showed an increase in IL-6 after FFP transfusion, the other

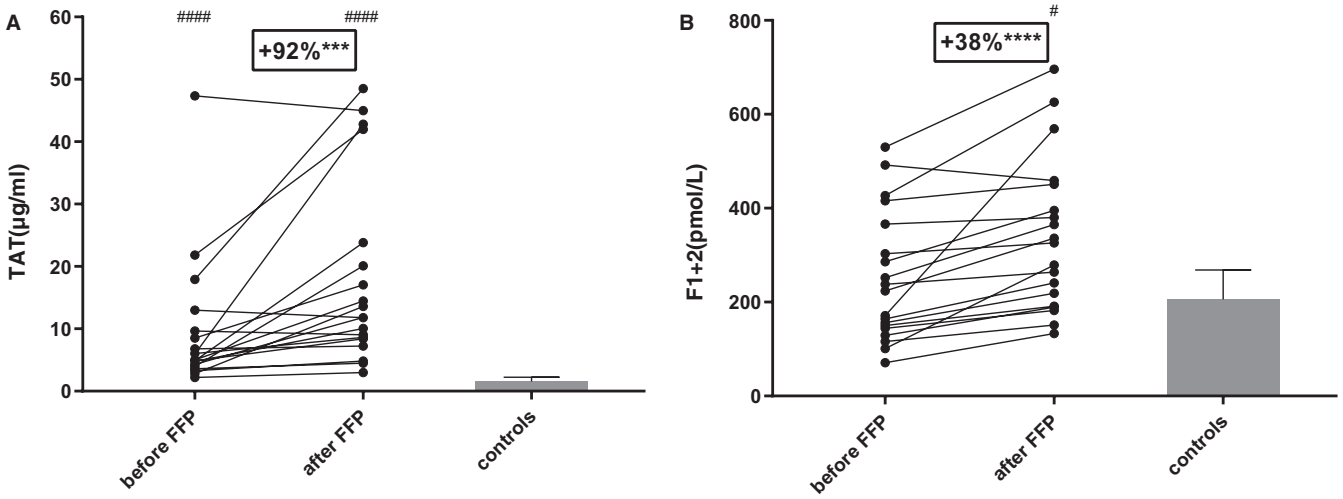

FIGURE 3 Plasma levels of markers for in vivo activation of coagulation, (A) TAT and (B) F1 + 2, were determined in patients with liver disease and a prolonged INR (n = 19) before and after FFP transfusion, and compared with plasma levels found in healthy controls (n = 20). Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. ***$P < .001$, ****$P < .0001$ before vs after FFP transfusion, #$P < .05$, ####$P < .0001$ patients vs controls. F1 + 2, prothrombin fragment 1 + 2; FFP, fresh frozen plasma; INR, international normalized ratio; TAT, thrombin/antithrombin complexes

six patients showed a decrease. CRP was substantially higher in patients at baseline, and levels decreased by 10 [2-22]% after FFP transfusion.

3.6 | No indication for NET formation following FFP transfusion

Levels of cell-free DNA and MPO-DNA complexes were higher in patients with chronic liver disease compared with healthy controls, and did not change after transfusion of FFP (Figure 4C,D).

3.7 | Platelet counts increase after platelet transfusion

We determined platelet counts before and after prophylactic platelet transfusion. Platelet counts increased by 43 [5-88]% from 28 [21-41] × 10^9/L before to 43 [39-64] × 10^9/L after platelet transfusion ($P < .01$) (Figure 5A). Of the 13 patients, six (46%) had a change in platelet count of ≤5 × 10^9/L. Four patients had an increase in count between 12 and 26 × 10^9/L, and three patients had an increase between 35 and 46 × 10^9/L. Notably, patients (n = 6) that were admitted to the liver intensive care unit (ICU) had a significantly higher increase in platelet count compared with patients (n = 7) that were admitted to the liver wards (31 [21-45] vs 2 [−1 to −5] × 10^9/L, $P < .01$).

3.8 | In vivo *activation of platelets after platelet transfusion*

Platelet activation was assessed by plasma levels of soluble CD40 Ligand, which is shed from platelets upon activation (Figure 5B). Plasma levels of soluble CD40 Ligand significantly increased by 15 [4-164] % from 131 (45-708) pg/mL before to 401 [59-835] pg/mL after platelet transfusion. Levels of soluble CD40 Ligand were lower in controls (52 [17-129] pg/mL) compared with patients, although the difference at baseline did not reach statistical significance ($P = .09$).

3.9 | Levels of TAT, but not F1 + 2, significantly increase after platelet transfusion

In vivo activation of coagulation was assessed by TAT and F1 + 2. Levels of TAT increased by 26 [4-33] % from 15.2 [8.3-20.5] µg/mL before to 17.6 [8.2-31.0] µg/mL after platelet transfusion ($P < .05$). Plasma levels of F1 + 2 did not change by platelet transfusion (before 496 [311-809] vs after 622 [306-1078] pmol/L). Plasma levels of both TAT and F1 + 2 were higher in patients compared with controls, as shown in Figure 6. Notably, the two patients that showed the largest percentual increases in levels of TAT and F1 + 2 were both patients who had their second blood sample taken after the procedure.

3.10 | Increased levels of IL-6 following platelet transfusion

Plasma levels of IL-6 and CRP were determined to assess a possible inflammatory response to platelet transfusion (Figure 7A,B). Levels of IL-6 increased by 9 [1-162]% following platelet transfusion from 27.9 [12.0-76.2] pg/mL to 58.8 [24.2-243] pg/mL. CRP did not change following platelet transfusion. Both markers of inflammation were higher in patients compared with controls.

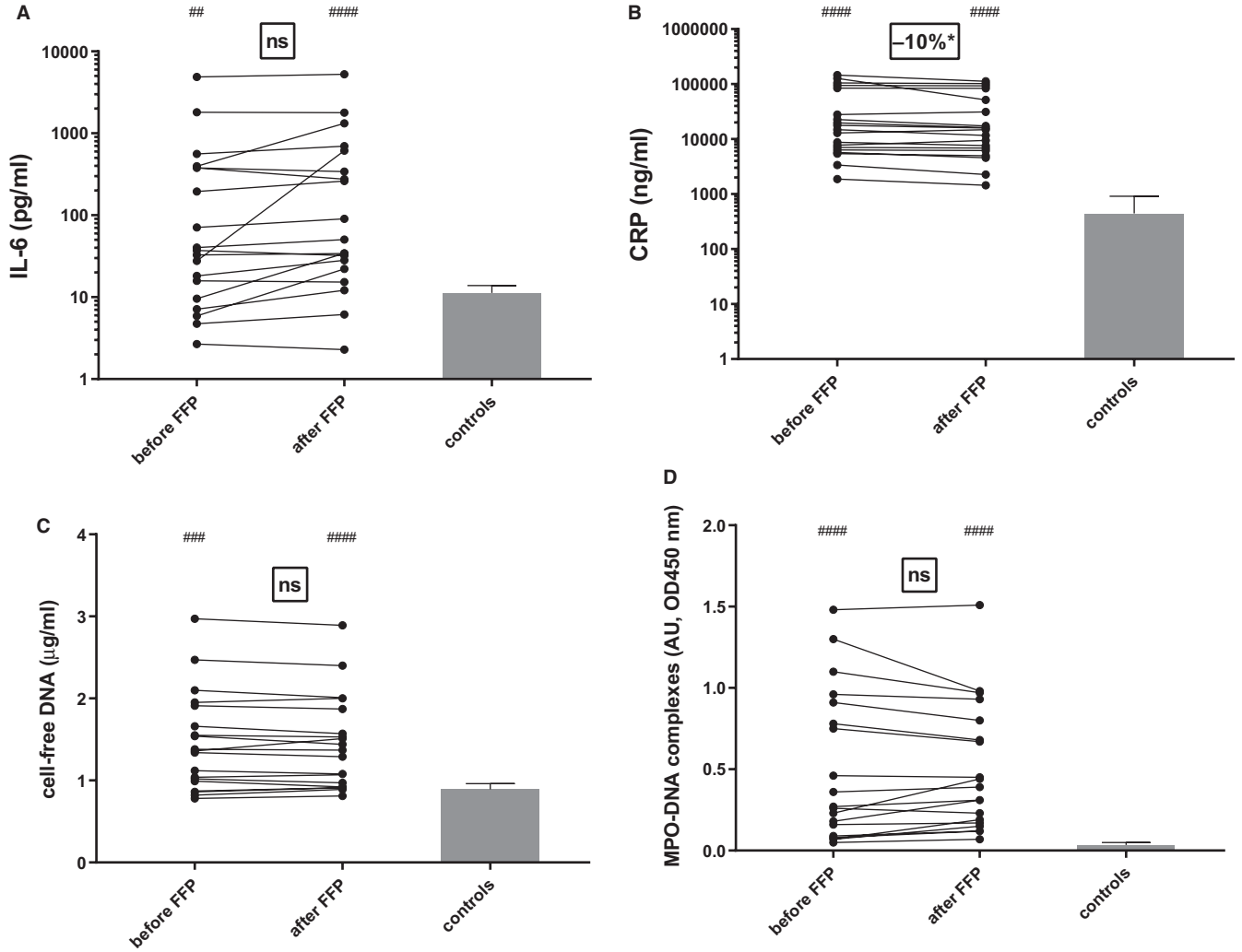

FIGURE 4 Inflammatory response to FFP transfusion was assessed by plasma levels of (A) IL-6 and (B) CRP. NET formation was assessed by plasma levels of (C) cell-free DNA and (D) MPO-DNA complexes. Blood samples from patients with liver disease and a prolonged INR (n = 19) were taken before and after FFP transfusion, and compared with plasma levels found in healthy controls (n = 20). Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. $^{*}P < .05$, before vs after FFP transfusion, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ patients vs controls. AU, arbitrary units; CRP, C-reactive protein; FFP, fresh frozen plasma; IL-6, interleukin-6; MPO, myeloperoxidase; NET, neutrophil extracellular trap; ns, not significant

3.11 | No indication of substantial NET formation following platelet transfusion

Plasma levels of markers for NETs were significantly higher, up to 10-fold for MPO-DNA complexes, in patients in comparison to healthy controls. Following platelet transfusion, levels of cell-free DNA were not different from levels before transfusion (Figure 7C,D). MPO-DNA complex levels slightly increased from 0.13 [0.09-0.89] AU to 0.19 [0.12-0.93] AU after platelet transfusion, $P = .059$.

3.12 | Bleeding and thrombotic complications

None of the patients that were included in this study had bleeding complications during or 1 day following the procedure. One patient that received a platelet transfusion was diagnosed with deep venous thrombosis after transfusion. However, it is likely that the thrombosis was preexistent because the patient was symptomatic before transfusion.

4 | DISCUSSION

In this study, the effect of prophylactic FFP and platelet transfusion on the hemostatic status of patients with liver disease was assessed in a real-life clinical setting. Following FFP transfusion, both in and ex vivo thrombin generation was significantly increased to levels higher than those found in healthy controls. These findings in part deviate from a recent clinical study in patients with chronic liver disease, where much more moderate increases in thrombin generation (5.7% and 23.4% for ETP and thrombin peak, respectively) following FFP transfusion were shown.[17] In line with previous studies,[18] platelet

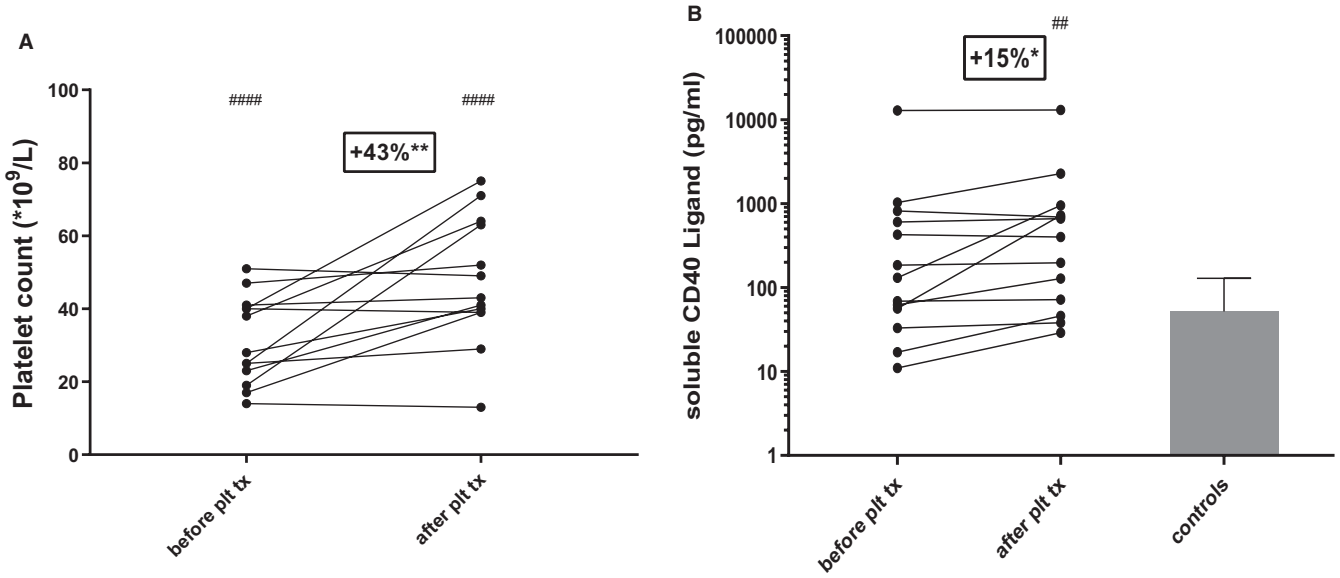

FIGURE 5 Platelet counts (A) in patients with liver disease-associated thrombocytopenia (n = 13) before and after platelet concentrate transfusion. In vivo platelet activation was assessed by plasma levels of (B) soluble CD40 Ligand before and after platelet transfusion, and compared with levels found in healthy controls (n = 20). Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. $^{*}P < .05$, $^{**}P < .01$ before vs after platelet transfusion, $^{\#\#}P < .01$, $^{\#\#\#\#}P < .0001$ patients vs healthy controls (of which platelet counts are not shown in graph A: (244 [184-323] $*10^9$/L)). plt tx, platelet transfusion

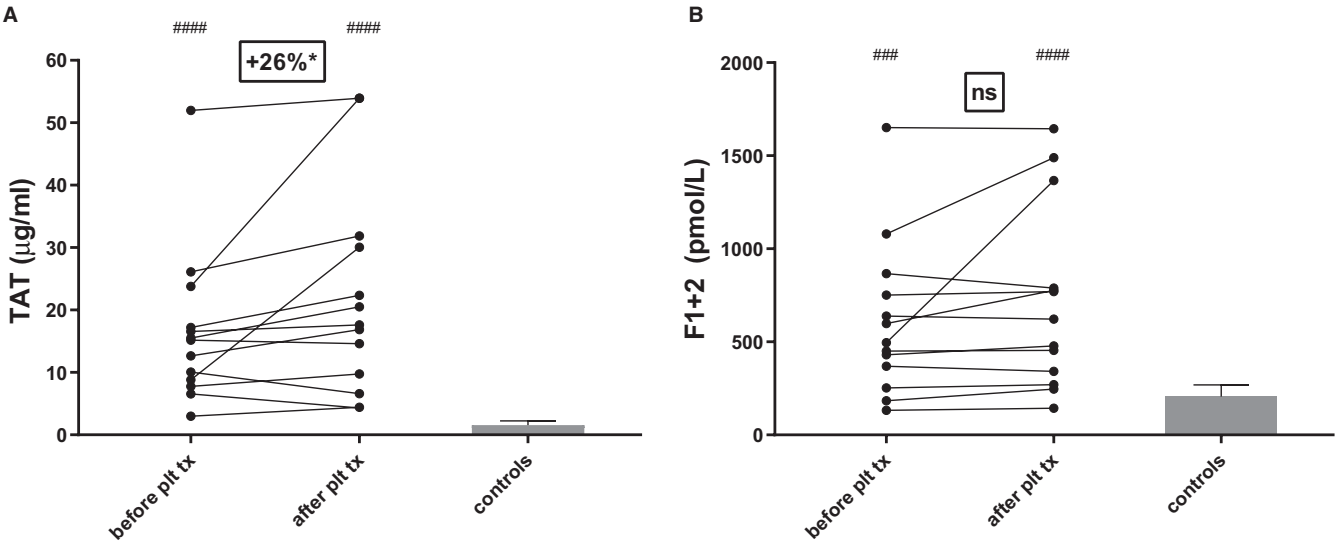

FIGURE 6 Plasma levels of markers for in vivo activation of coagulation, (A) TAT and (B) F1 + 2, were determined in patients with a liver disease-associated thrombocytopenia (n = 13) before and after platelet transfusion, and compared with plasma levels found in healthy controls (n = 20). Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. $^{*}P < .05$, before vs after platelet transfusion, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ patients vs controls. F1 + 2, prothrombin fragment 1 + 2; ns = not significant; plt tx, platelet transfusion; TAT, thrombin/antithrombin complexes

transfusion resulted in a median increase in platelet count of 15 × 10^9/L, corresponding with a platelet count of 43 [39-64] × 10^9/L after platelet transfusion, which was accompanied by increases in in vivo markers of coagulation activation, and IL-6, and by a slight increase in a marker for NET formation.

As expected, conventional coagulation tests improved after FFP transfusion. However, most of the patients receiving FFP still had INR >1.5 after transfusion, a preprocedure threshold that is still used by many proceduralists in clinical practice. Thrombin generation parameters increased substantially after FFP transfusion in the majority of the patients, indicating a prothrombotic effect of FFP transfusion. However, the ETP in patients before FFP transfusion was already comparable to ETP in healthy controls, suggesting that thrombin generation in patients

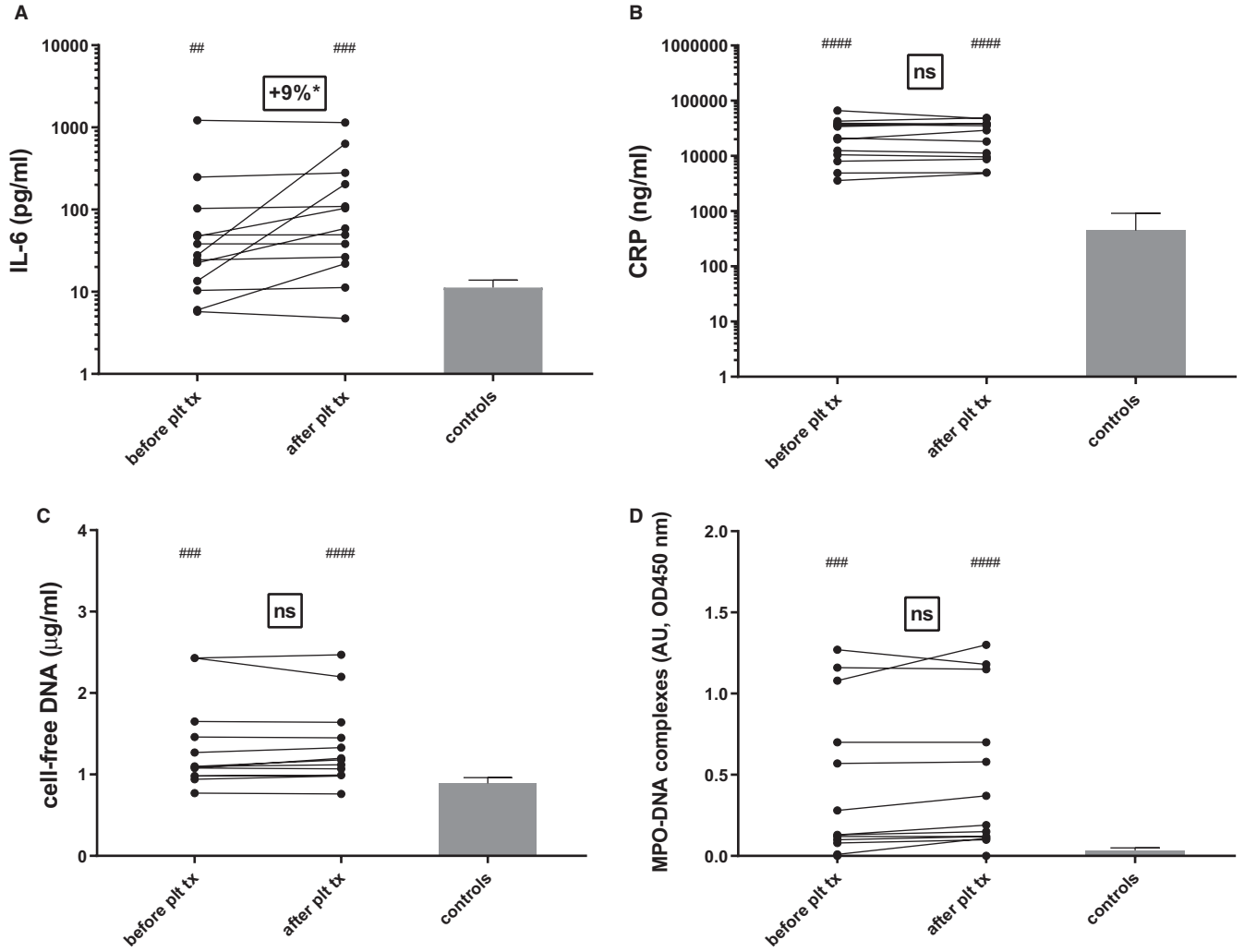

FIGURE 7 Plasma levels of markers for inflammation, (A) IL-6 and (B) CRP, were determined in patients with a liver disease-associated thrombocytopenia (n = 13) before and after platelet transfusion, and compared with plasma levels found in healthy controls (n = 20). NET formation was assessed by plasma levels of (C) cell-free DNA and (D) MPO-DNA complexes in patients before and after platelet transfusion and in controls. Dots representing individual patient data before transfusion are connected by a line to data after transfusion. Bars represent median ± interquartile ranges. $^{*}P < .05$, before vs after platelet transfusion, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ patients vs controls. AU, arbitrary units; CRP, C-reactive protein; IL-6, interleukin-6; MPO, myeloperoxidase; NET, neutrophil extracellular trap; ns, not significant; plt tx, platelet transfusion

was preserved before transfusion. These patients thus received FFP transfusion for their presumed coagulopathy, based on their high INR, but based on thrombin generation potential prohemostatic therapy would not have been indicated. We have no clear explanation for the discrepancy in increase of the ETP following FFP transfusion between our study and the recently published study mentioned previously,[17] but do note the sample sizes of both studies were limited. In addition, the thrombin generation test has substantial laboratory-to-laboratory variability[27] that may explain some of the differences between the studies, and combined with the small sample size of this study comprise a considerable limitation that warrants cautious interpretation of these results. We also demonstrated that in vivo markers of coagulation activation (TAT and F1 + 2) significantly increased following FFP transfusion, which confirms a prothrombotic effect of FFP transfusion in most of the patients in our cohort. However, because baseline hemostatic status as assessed by thrombin generation was already normal in our patients, the increases in TAT and F1 + 2 after FFP transfusion could indicate increased thrombotic risk. Importantly, FFP transfusion did not have a prothrombotic effect in a substantial proportion of the patients. This variable response to FFP transfusion further challenges its use and underlines the importance of identifying which patients would benefit from transfusion.

Platelet counts increased from 28 before to 43 × 10^9/L after transfusion. Approximately two-thirds of the patients still had platelet counts <50 × 10^9/L after transfusion, a platelet count that is frequently targeted before procedures in liver disease patients. There was a striking difference in the increase in platelet count between patients who were admitted to the liver ICU and those on the liver

wards (31 [21-45] vs 2 [−1 to −5] × 10^9/L). Given the small sample size of this study, it is difficult to distinguish whether this is a true or random finding. Possible explanations, such as timing of the second blood sample and spleen size, were considered, but did not appear to account for the difference (data not shown). Levels of the platelet activation marker soluble CD40 ligand significantly increased after platelet transfusion, although not in all patients, suggesting increased in vivo activation of platelets following platelet transfusion. An alternative explanation could be that part of the platelets that were infused were already activated in the transfusion bag before infusion.[28] Future studies should assess potential changes in ex vivo platelet function following platelet transfusion to ascertain whether the increase in platelet count increases hemostatic potential. Our finding that following platelet transfusion, plasma levels of TAT, but not F1 + 2, increased in part of the patients may indicate a true prothrombotic effect of platelet transfusion. Notably, three of the 13 blood samples after platelet transfusion were taken after the procedure, which could potentially have contributed to the increase in plasma levels of TAT. However, there was no difference in levels of TAT and F1 + 2 in blood samples that were taken before or after the procedure.

Most of the procedures that patients in this cohort underwent were associated with a low or intermediate bleeding risk, where current clinical guidance now advises against the use of prophylactic administration of blood products.[8-10,14] It is important to note that the patients we studied represented only a small proportion of those undergoing such procedures, many of whom received no such support. Nonetheless, even in a specialized liver care center, some clinicians continue to administer blood products to selected patients with liver disease prior to low- or moderate-risk procedures based on abnormal conventional coagulation tests. This illustrates the challenges in changing clinician behaviors of long standing, even when there is evidence that prophylactic transfusions are potentially harmful.[20-22] For example, transfusion of FFP may lead to volume overload and may exacerbate portal hypertension that could increase rather than prevent bleeding risk in these patients.[6] Here, we demonstrate potential prothrombotic effects of blood product transfusion. In addition, we found laboratory evidence of a potentially harmful inflammatory effect of platelet transfusion, which is in accordance with a previous study from our group that assessed inflammatory responses following platelet transfusion.[29] Adverse effects of blood product transfusion should be an integral part in the decision making of prophylactic blood product transfusion in patients with liver disease.

In conclusion, administration of FFP and platelet transfusion in a real-life clinical setting resulted in a prothrombotic effect in some but not all patients with chronic liver disease. However, many of the transfusions were not indicated based on recent guidance documents, and it is questionable whether these transfusions had a clinically relevant effect. Our results do suggest that blood product transfusions may be useful in improving hemostatic status in some high-risk procedures or actively bleeding patients.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Concept and design: Fien A. von Meijenfeldt, Bente P. van den Boom, Lara N. Roberts, Ton Lisman, William Bernal. Data acquisition: Fien A. von Meijenfeldt, Bente P. van den Boom, Jelle Adelmeijer. Data interpretation: all. Manuscript drafting: Fien A. von Meijenfeldt, Ton Lisman, William Bernal. Revision of manuscript: all. Obtained funding: Fien A. von Meijenfeldt, Ton Lisman. Study supervision: Ton Lisman, William Bernal.

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