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ORIGINAL PAPER

Endothelial activation predicts disseminated intravascular coagulopathy, cytokine release syndrome and prognosis in patients treated with anti-CD19 CAR-T cells

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Summary
Cytokine release syndrome (CRS) and consumptive coagulopathy can complicate the treatment with chimeric antigen receptor T (CAR-T) cells. The modified version of the Endothelial Activation and Stress Index (mEASIX), a score derived from haematopoietic stem cell transplantation, combines platelets, C-reactive protein (CRP), and lactate dehydrogenase (LDH) and has been correlated with CRS and endothelial biomarkers. In 38 consecutive patients with aggressive lymphoproliferative disease we measured a coagulative laboratory panel at baseline and early after infusion of anti-CD19 CAR-T. The panel was investigated also in the presence of CRS graded 2 or higher, or immune effector cell-associated neurotoxicity syndrome (ICANS). Moreover, we examined the relationship between mEASIX, coagulation biomarkers, and toxicities of CAR-T cells. During CRS grade 2 or higher, we found increased prothrombin time (PT) and activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, factor VIII (FVIII), and von Willebrand factor (vWF) antigen levels, and decreased platelet count and antithrombin levels. The occurrence of immune effector cell-associated neurotoxicity syndrome was associated with higher PT values, D-dimer, FVIII, and vWF levels, and decreased fibrinogen levels and platelet count. A higher mEASIX score correlated with increased aPTT values, fibrinogen, D-dimer, FVIII and vWF levels, and decreased antithrombin levels. Baseline mEASIX was predictive for consumptive coagulopathy and CRS graded 2 or higher, and for progression-free survival and overall survival.

KEYWORDS
CAR-T cells, cytokine release syndrome, disseminated intravascular coagulation, endothelium activation, immune neurotoxicity syndrome, non-Hodgkin lymphoma

INTRODUCTION

In recent years, chimeric antigen receptor T (CAR-T) cells have revolutionized the therapeutic approach to several lymphoid malignancies.[1,2]

The most common CAR-T cell-related toxicities are the cytokine release syndrome (CRS) and the immune effector cell-associated neurotoxicity syndrome (ICANS).[1] CRS is a systemic hyperinflammatory response, characterized by fever, hypoxia, hypotension, and rarely by organ failure.

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CRS is the most frequent acute toxicity related to CAR-T cells. A recent real-world study on 1297 patients treated with axicabtagene ciloleucel (axi-cel) reported CRS of any grade in 83% of cases, with grade 3 or more in 8%.[2] The main mediators of CRS are pro-inflammatory cytokines, such as interferon gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1, or IL-6. When CAR-T cells recognize a tumour antigen, they activate pyroptosis, a type of programmed death mediated by perforin, granzymes, and caspase.[3] Tumour cell death releases inflammatory soluble factors, which mediate effects per se or activate macrophages via Toll-like receptor pathways and specific kinases such as mitogen-activated protein kinase (MAPK).[4] In turn, IL-6 activates the JAK and STAT3 pathways, inducing endothelial cells to secrete pro-inflammatory molecules such as vascular-endothelial growth factor (VEGF), IL-8, and plasminogen activator inhibitor PAI-1.[5,6] Those mechanisms may lead to loop amplification, and often require a pharmacological target intervention with anti-IL-6-receptor/IL-6/IL-1 monoclonal antibodies, or corticosteroids.

Patients with CRS show higher prothrombin time (PT) and activated partial thromboplastin time (aPTT) values, higher D-dimer levels, and lower fibrinogen levels and platelet counts.[7,8] Moreover, patients with CRS grade 4–5 have higher expression of endothelial biomarkers than those with milder CRS, showing a higher peak of von Willebrand factor (vWF), soluble endothelial selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), and angiopoietin (Ang)-2 and lower Ang-1.[9]

Immune effector cell-associated neurotoxicity syndrome occurs in hyperinflammatory scenarios: VEGF, thrombin, and epinephrine mediate the activation of endothelial cells, leading to augmented secretion of Ang-2 with an overbalance on Ang-1. Consequently, the permeability of the blood–brain barrier becomes impaired and contributes to the pathogenesis of ICANS.[4,10,11] Patients with ICANS grade 4 have a lower fraction of high-molecular-weight vWF, possibly due to consumption, and a lower ADAMTS13 in comparison with patients with ICANS of grade 3 or less, with a mechanism similar to that of microangiopathic syndromes.[10] Thus, both CRS and ICANS are deeply connected to coagulative alteration and endothelial activation.

In patients undergoing allogeneic stem cell transplantation, the Endothelial Activation and Stress Index (EASIX) is an easy bedside score, which considers platelets, creatinine, and lactate dehydrogenase (LDH), and can mirror the endothelial activation and damage.[12]

EASIX is strictly associated with the development of graft versus host disease (GVHD) and post-transplant microangiopathies. Moreover, EASIX predicts the outcome of COVID infection[13] and the development of fluid overload[14] or sepsis[15] related to allogeneic stem cell transplantation, confirming the ability to mirror the endothelial stress and impairment.

In consideration of the moderate role of alterations of creatinine in the early phase after CAR-T cell infusion, and that inflammation is the leading phenomenon during the first 10 days, a modified version of EASIX (mEASIX), with C-reactive protein (CRP) replacing creatinine, has been proposed in the CAR-T cell setting.[16]

Two studies have shown a positive correlation between mEASIX and serum biomarkers associated with endothelial activation and distress[16,17]; mEASIX was associated with the occurrence of any grade of CRS, and with severe CRS. Post-infusion (but not pre-infusion) assessment of mEASIX was associated with any grade of ICANS and with severe ICANS.[16]

Overall, endothelial impairment is emerging as highly involved in the scenario of acute toxicities related to cell therapies; at the same time, those toxicities have been described to associate with the activation of patterns of intravascular coagulation. In this study, we aimed to explore the impact and mutual association of hypercoagulation, acute toxicities, and endothelial impairment expressed with mEASIX in the setting of the early post-CAR-T phase.

METHODS

Patients

All consecutive patients treated with anti-CD19 CAR-T cells between September 2019 and August 2022 at our department were included. The lymphodepletion regimen consisted of fludarabine and cyclophosphamide over three days for all patients, with dosages as per product schedule.

We included 38 consecutive patients, mostly affected by aggressive B-cell lymphomas (92%, see Table 1). The median follow-up was 186 days. Patients had received two median lines of therapy, mainly including R-CHOP (74%), platinum-based salvage regimens (71%), and autologous haematopoietic stem cell transplantation (ASCT). Eleven (29%) patients with aggressive B-cell lymphoma had received ASCT before inclusion in the CAR-T cell programme, while eight (21%) more patients had received ASCT as bridging therapy.

Three (8%) patients had received no bridging therapy, while the remaining participants received either localized radiotherapy (31%), other chemotherapy-based regimens (38%), or biological treatments (23%), mainly with ibrutinib or lenalidomide. The CAR-IL-T cell products employed were axicabtagene ciloleucel (axi-cel), brexucabtagene autoleucel (brexu-cel), and tisagenlecleucel (tisa-cel) in 45%, 16% and 39% of patients respectively.

When the lymphodepletion regimen was administered, 44% of patients were receiving prophylactic parenteral anticoagulation with low-molecular-weight heparin (LMWH), mainly due to tumoural bulk or disease-dependent immobilization. Five (13%) more patients were receiving LMWH at a therapeutic dosage due to a previous thrombosis, while four (10%) patients were treated with antiplatelet agents for cardiovascular comorbidities. Antiplatelet agents and LMWH were administered for platelet counts higher than 50×10^9/μl or 30×10^9/μl respectively.

TABLE 1 Patient characteristics at inclusion in the CAR-T programme

Age	
Median	50 (28–75)
Gender	
Female	20(53%)
Male	18 (47%)
Previous lines	
Median	2 (2–7)
Previous autologous SCT	11 (29%)
Previous allogeneic SCT	2 (5%)
IPI[a]	
0–1	11 (32%)
2	9 (26%)
3	8 (23%)
4–5	5 (15%)
CAR-T product	
Axicabtagene ciloleucel	17(45%)
Tisagenlecleucel	15 (39%)
Brexucabtagene autoleucel	6 (16%)
Diagnosis	
Diffuse large B-cell lymphoma	20 (53%)
Transformed follicular lymphoma	7 (18%)
Mantle cell lymphoma	3 (8%)
Primary mediastinal B-cell lymphoma	5 (13%)
Acute lymphoblastic leukaemia	3 (8%)
Bridging therapy	
None	3 (8%)
Biological/immunotherapy	8 (23%)
Radiotherapy	12 (31%)
Chemotherapy	15 (38%)
State of disease prior to CAR-T	
CR/PR	14 (37%)
SD/PD	19 (63%)
Antiaggregation/anticoagulation during CAR-T[b]	
None	13 (33%)
Prophylactic anticoagulation	17 (44%)
Therapeutic anticoagulation	5 (13%)
Antiaggregation	4 (10%)

Abbreviations: CAR-T, chimeric antigen receptor T; CR, complete response; IPI, International Prognostic Index; PD, proliferating disease; PR, partial response; SCT, stem cell transplantation; SD, stable disease.

[a]IPI was calculated only for patients with lymphoma.

[b]One patient was under both anti-aggregating and anti-coagulant therapy when beginning treatment with CAR-T cells, and was consequently listed in both categories in this table.

The patients' characteristics are shown in Table 1.

The study was conducted according to the Helsinki criteria and was approved by the local ethics committee (ID 4879 Prot 0020777/22). Patients provided informed signed consent for biobanking and anonymized use of data.

Procedures

We designed a panel for acute inflammation, coagulative asset, and endothelial activation. The panel included the measurement of platelet counts, antithrombin, PT, and aPTT values, and the dosage of D-dimer, fibrinogen, vWF antigen, factor VIII (FVIII) activity, ferritin, CRP, IL-6, IL-2R, and LDH levels.

All patients were tested at baseline before lymphodepletion, immediately before CAR-IL-T cell reinfusion, and every other day after CAR-IL-T cell reinfusion for the first two weeks.

The mEASIX was calculated as follows: CRP (mg/L) × LDH (U/L)/platelet count ($\times 10^9$/μl). A $\log_2$ transformation was then applied to reduce skewness.

Disseminated intravascular coagulation (DIC) was identified with the score proposed by the International Society on Thrombosis and Haemostasis (ISTH)[18]: in that system, platelet count, fibrinogen, PT, and fibrin-related markers are assigned zero to three points, and a condition with a score of 5 or more is considered compatible with overt DIC. The modified EASIX was calculated for every time-point; the occurrence of CRS and ICANS was explored daily and events were graded according to current American Society for Transplantation and Cellular Therapy (ASTCT) criteria.[19] Infections and other toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 scoring system.

While grade 1 CRS normally is characterized by isolated fever, higher grades of CRS tend to present with hypoxia and/or hypotension, as a possible counterpart of an endothelial systemic involvement. Therefore, we have compared coagulative markers determined when CRS was absent or mild (grade 0–1) with determinations made when moderate-to-severe CRS was active (grade 2–5). Similarly, we analysed the same biomarkers when any grade ICANS was active (grade 0 vs grade 1–5). The efficacy outcomes of CAR-T cells were progression-free survival (PFS) and overall survival (OS). PFS and OS were calculated starting from CAR-T cells reinfusion.

Statistical analysis

All continuous variables were analysed for normality and variances with the Shapiro–Wilk test and the equal-variances modified Levene test. Continuous variables were compared by categorical variables: if distribution of continuous variables was normal, we applied either the equal-variances *T*-test or the Aspin–Welch unequal-variance *T*-test, if variances between groups were equal or not respectively. If the distribution was not normal, the Mann–Whitney test was preferred. To measure the strength of the relationship between each coagulative parameter and mEASIX, CRS and ICANS, and compute their association, Pearson correlation or Spearman rank correlation tests (according to the normality of distribution) were applied. The predictivity of mEASIX at D0 for D4 coagulopathy was tested with a receiver operating

TABLE 2 Coagulation parameters in patients without or -with CRS and ICANS

	Distribution[a]	CRS 0–1		CRS 2–5		Variances[b]		ICANS 0		ICANS 1–5		Variances[b]	
Variable	Normal	Median	95% CI	Median	95% CI	Equal	*p*	Median	95% CI	Median	95% CI	Equal	*p*
PT (s)	No	11.4	11.3–11.9	12.5	11.5–12.9	No	0.018	11.5	11.4–11.9	12.9	11.9–17.7	No	<0.001
aPTT (s)	No	31	30–32.1	34	33–35.9	Yes	<0.001	31.9	30.6–32.7	31.4	28.6–34.7	Yes	0.88
FBG mg/dl	No	336	304–371	499	364–560	No	<0.001	368	332–402	216	169–433	Yes	0.01
D-Dimer ng/ml	No	1319	1084–1652	2220	1249–5538	No	0.002	1249	1102–1652	5784	1965–12 699	No	<0.001
PLT x10^9/µl	No	87	76–97	55	46–66	No	<0.001	82	73–92	26	24–46	No	<0.001
AT (%)	Yes	93	88–95	84	82–89	Yes	0.009	92	87–94	90	82–102	Yes	0.96
FVIII (%)	Yes	184	175–192	225	196–256	Yes	0.002	187	178–196	232	172–321	Yes	0.015
vWF iu/dl	Yes	208.1	189–226	286	257–332	Yes	<0.001	213.8	197.7–239.9	308.4	204.7–394.5	No	0.015
mEASIX	Yes	5.76	4.9–6.3	8.98	8.11–9.58	No	<0.001	6.23	5.65–6.52	8.14	7.25–9.78	Yes	<0.001

Abbreviations: aPTT, activated partial thromboplastin time; CI, confidence interval; CRS, cytokine release syndrome; FBG, fibrinogen; FVIII, factor VIII; ICANS, immune effector cell-associated neurotoxicity syndrome; mEASIX, modified Endothelial Activation and Stress Index; PLT, platelet; PT, prothrombin time; vWF, von Willebrand factor.

[a]Normality of distribution was assessed with the Shapiro–Wilk test.

[b]Equality of variances was assessed with the modified Levene test.

characteristic (ROC) analysis. PFS and OS were calculated with the Kaplan–Meier log-rank test. Multivariate analyses for survival analysis were performed with Cox regression tests. Tests were performed with NCSS 2020 Statistical Software (2020) LLC., Kaysville, UT, USA.

RESULTS

Adverse events

All patients experienced CRS during the first 10 days after CAR-T cell reinfusion. In 30 patients (79%) CRS was grade 2 or more, being fever associated with hypotension and/or hypoxemia. The median onset of CRS grade 2 was on day 3 after CAR-T reinfusion, and the median duration was five days. ICANS occurred in 12 (31%) patients. In four patients (10%) ICANS was grade 3–4. The median onset of ICANS was on day 5 and the median duration was three days (range 1–20). No grade 5 toxicity was recorded. Details on CRS, ICANS, and their treatment are shown in Table S1.

Overall, 26 (68%), seven (18%), and two (5%) patients received treatment for CRS or ICANS with tocilizumab, steroids and vasopressors respectively. The management of toxicities was conducted according to current EBMT/JACIE/EHA guidelines.[20]

Nine (24%) patients had grade 3–4 infections, requiring parenteral antibiotic therapy and/or potentially life-threatening: seven cases had a bacterial aetiology (four bloodstream infections, two pneumonia, one cystitis), and two were viral infections (one cytomegalovirus reactivation and one rhinovirus infection).

No patient experienced a major bleeding; one patient had a symptomatic catheter-related deep-vein thrombosis (DVT) with pulmonary embolism (PE).

Disseminated intravascular coagulation

Patients with CRS grade 2 or higher had prolonged PT and aPTT values, a lower platelet count, and decreased antithrombin levels in comparison with those with CRS mild or absent. Moreover, in moderate-to-severe CRS we have observed fibrinogen, D-dimer, FVIII, and vWF antigen levels higher than in mild or absent CRS. Detailed information on biomarkers and coagulation assessment according to the presence of CRS is provided in Table 2. At baseline, immediately before reinfusion of CAR-T cells, all but one patient had an ISTH score lower than 5. Conversely, if assessed while experiencing CRS grade 2 or more, 30% of the evaluable patients presented an ISTH score of 5 or more, consistent with DIC.

Moreover, we analysed coagulation biomarkers for patients experiencing ICANS, compared to samples collected in absence of ICANS. When ICANS was present, patients showed increased PT values, with no difference in aPTT values nor in antithrombin levels, and a lower platelet count. Patients with active ICANS had higher D-dimer, FVIII, and

vWF antigen levels, and lower fibrinogen levels than those without ICANS. Full details on coagulation and ICANS are provided in Table 2.

Endothelial activation and DIC/CRS/ICANS

Additionally, we focused on the relationship between coagulation, endothelial damage, CRS, and ICANS during the first two weeks after CAR-T cell infusion.

Median mEASIX at baseline was 5.05 [95% confidence interval (CI) 4.01–5.99]. A baseline mEASIX higher than 6.43 was predictive for the development of CRS grade 2 or higher [ROC analysis, sensitivity 43% specificity 100%, area under the curve (AUC) 67%, $p = 0.03$] during the first two weeks after CAR-T cell infusion. After CAR-T cell infusion, patients with no/grade1 CRS had lower mEASIX (median 5.76, 95% CI 4.0–6.3) compared to patients with any CRS of grade 2 or higher (median 8.99, 95% CI 8.11–9.58; $p < 0.001$).

Similarly, patients with no ICANS had lower mEASIX compared to patients with ICANS (median 6.23, 95% CI 5.65–6.52, vs median 8.14, 95% CI 7.2–9.7 respectively; $p < 0.001$). Still, the mEASIX value at day 0 was not predictive for ICANS in a dedicated ROC analysis ($p = 0.1$).

Higher mEASIX was associated with laboratory findings suggesting an active coagulative process and endothelial involvement. There was a positive correlation between mEASIX and aPTT ($p < 0.001$, $R = 0.38$), fibrinogen ($p < 0.001$, $R = 0.35$), D-dimer ($p < 0.001$, $R = 0.39$), factor VIII ($p = 0.007$, $R = 0.21$), and vWF ($p < 0.001$, $R = 0.39$), and a negative correlation with antithrombin ($p < 0.001$, $R = -0.36$) (Figure 1). We have also found a positive correlation with sST2 (soluble Suppression of Tumorigenicity 2) and suPAR (soluble urokinase-type plasminogen activator receptor), despite data being available for a limited number of patients (Figures S1 and S2). We have only found a positive trend between mEASIX and PT ($p = 0.078$, $R = 0.17$). As platelet count is in the denominator of the mEASIX formula, their association was not explored.

Overall, 13/38 (34%) patients fulfilled ISTH criteria for DIC during the first two weeks after CAR-T cell infusion. Overt DIC was strongly associated with simultaneous mEASIX scoring. Moreover, we explored by a ROC analysis if baseline mEASIX was predictive for the development of DIC: we found that a value of mEASIX higher than 6.89 predicted DIC during the two weeks after infusion. (ROC analysis, sensitivity 53%, specificity 88%, AUC 0.69, $p = 0.028$). Coagulopathy defined as per ISTH criteria occurred in 29%, 33% and 40% of patients treated with axi-cel, brexu-cel and tisa-cel respectively ($p = 0.8$).

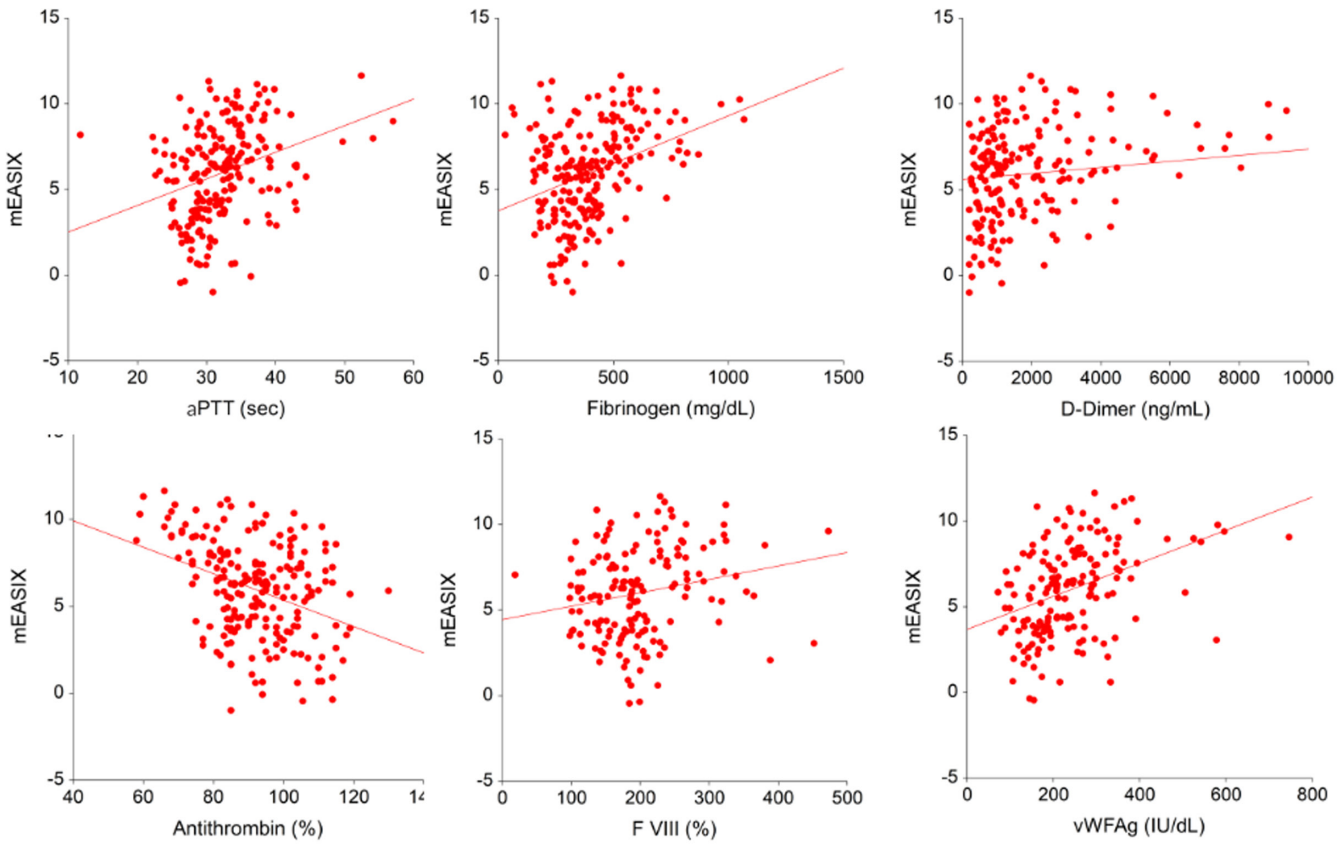

FIGURE 1 Correlation between the modified Endothelial Activation and Stress Index (mEASIX) score and major parameters for consumptive coagulopathy. The plots highlight a positive correlation with activated partial thromboplastin time values, fibrinogen, D-dimer, FVIII and vWF antigen levels, and a negative correlation with antithrombin levels. *p* and *R* values are provided in the text.

mEASIX and prognosis

When considering the ROC analysis for CRS and DIC, cut-off values of baseline mEASIX of 6.43 and 6.89 respectively predicted the occurrence of toxicities. Therefore, we opted for the cut-off mEASIX of 6.89 for estimating PFS and OS. We observed no death due to toxicities or complications that occurred after CAR-T treatment. All deaths were due to progression. As median follow-up was 186 days, we set our analysis at day 180. PFS at day 180 after CAR-T cell infusion was 60% vs 33% for patients with a baseline mEASIX lower or higher than 6.89 respectively ($p = 0.031$) (Figure 2). OS at day 180 was 90% vs 50% for patients with a baseline mEASIX lower or higher than 6.89 respectively ($p = 0.024$) (Figure 2).

We then performed a Cox regression multivariate analysis for survival outcomes considering baseline mEASIX, anytime development of DIC, CRS grade higher than 2, and ICANS. Baseline mEASIX, considered as a continuous variable, was the only independent predictive risk factor both for PFS and OS (data not shown). Baseline mEASIX with a cut-off value of 6.89 was the only independent predictive risk factor for PFS ($p = 0.024$), with a trend for predicting OS ($p = 0.054$) (Table S2). The odds ratios for PFS and OS in patients with baseline mEASIX higher than 6.89 were 4.95 and 6.14 respectively.

DISCUSSION

Cytokine release syndrome and DIC are associated in up to 56% of patients treated with CAR-T cells.[21,22] Many of these alterations seem to be common to different CAR-T constructs, independently of the viral vector (lentivirus or retrovirus), tumour antigen (CD19, CD20, BCMA), or costimulatory domain (CD137, CD28, or 41BB).[23–25] American Society of Clinical Oncology (ASCO) guidelines have recommended supportive therapy and factor replacement in case of DIC, on the basis of fibrinogen levels, aPTT, and occurrence of bleeding.[1] In Table 3 we provide an overview of recent findings on coagulative and endothelial biomarkers modifications early after infusion of CAR-T cells.

In the present study we found that fibrinogen levels were increased when active CRS was present. This was in partial disagreement with what was observed by many authors, as more often a decrease of fibrinogen is reported. Usually, fibrinogen is highly specific in predicting consumptive coagulopathy but shows low sensitivity in highly inflammatory conditions. According to this consideration, the Japanese Association for Acute Medicine's DIC diagnostic criteria (JAAM criteria) do not score fibrinogen for diagnosing DIC.[26]

We hypothesized that fibrinogen consumption was overcome due to opposite trends originating from activated consumptive coagulopathy and acute inflammatory state. Notably, we have observed an opposite trend when analysing fibrinogen in the presence or absence of ICANS. In this setting, the impact of the formation of microthrombi may be more relevant overcoming the inflammatory contribution to fibrinogen elevation. In this sense, given the same lymphodepletion, we have found that patients with higher CRS and ICANS showed lower platelets and higher D-dimer, suggesting an effective role for *in-vivo* micro-coagulative processes.

According to Jiang and colleagues, patients with any coagulative alteration after CAR-T cell treatment, fulfilled the Chinese DIC Scoring System (CDSS) for diagnosis of DIC in 50% of cases, mainly with no or minor mucocutaneous bleeding.[21] Bleeding has been reported in 2% of patients and is generally graded 3 or less.[7]

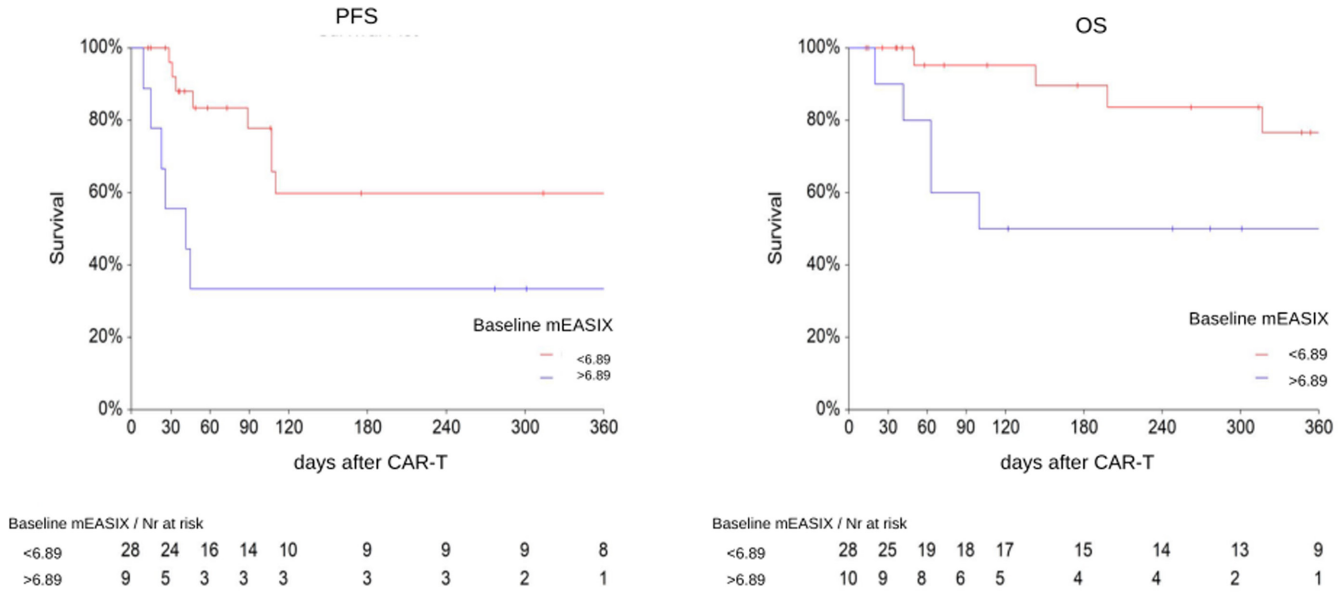

FIGURE 2 Kaplan–Meier curves with survival outcomes [progression-free survival (PFS) and overall survival (OS)] by baseline modified Endothelial Activation and Stress Index (mEASIX) score lower than 6.89 (red line) or higher than 6.89 (blue line). Median survival at 180 days and *p* values are provided in the text.

TABLE 3 Overview of coagulative parameters alterations according to major reports in patients treated with CAR-T cells

Changes after CAR-T cell infusion[a]	Patients	Target antigen/costimulatory demain	Platelets	FBG	TM	D-D	FDP	PT	aPTT	INR	AT	PAI-1	TAT
Gust J, 2017[10]	133	CD19/41BB	↓	↓		↑		↑	↑				
Hay KA, 2017[23]	133	CD19/41BB	↓	↓		↑		↑	↑				
Jiang H, 2019[21]	53	CD19/CD137	↓			↑	↑		↑				
Wang Y, 2020[8]	100	CD19, CD20, or BCMA/41BB		↓		↑			↑	↑			
Buechner J, 2021[7]	137	CD19/41BB	↓	↓				↑	↑				
Johnsrud A, 2021[24]	127	CD19 or CD19/CD22/CD28	↓	↓		↑		↑	↑				
Shao M., 2021[25]	37	BCMA	↓			↑		=	↑				
Yamasaki-Morita M, 2022[22]	25	CD19/41BB		↑	=	↑	↑		=	=	↓	↑	↑

Abbreviations: AT, antithrombin; aPTT, activated partial thromboplastin time; CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; D-D, D-dimer; FBG, fibrinogen; FDP, fibrin degradation products; ICANS, immune effector cell-associated neurotoxicity syndrome; PAI-1, plasminogen activator inhibitor 1; PT, prothrombin time; TAT, thrombin-antithrombin complex; TM, thrombomodulin.

[a]Coagulation parameters have been compared between baseline and after CAR-T cell reinfusion (Yamasaki-Morita et al. [22], Jiang et al. [21] and Wang et al. [8]), or comparing patients with or without CRS (Hay [23], Shao [25], Buechner [7]), ICANS (Gust et al. [10]) or bleeding (Johnsrud et al. [24]).

Both ISTH and CDSS scores consider PT, fibrinogen, D-dimer, and platelet count; still, in the CAR-T cells setting a low platelet count may be due to the previous fludarabine-based lymphodepletion, whereas fibrinogen reduction may be counterbalanced by the hyperinflammatory state of CRS. Therefore, classical DIC scoring systems may not be adequate for these patients, and further studies are needed to build a DIC score adapted to this setting.

Despite some limitations to our study, such as its retrospective nature, limited sample size, and determination of biomarkers every other day instead of daily for ethical reasons, we have confirmed the onset of consumptive coagulopathy in the post-infusion setting of CAR-T cells and we have validated a decrease of antithrombin, as recently reported.[22] The association between coagulopathy and CRS grade 2 or higher, when patients suffer also from hypotension or hypoxia, appears in keeping with systemic endothelial activation.

The process of endothelial activation leads to the release of Weibel Palade bodies. These bodies store two principal molecules, P-selectin and vWF, which play a key role in inflammation and haemostasis.[27] The main source of vWF, whose role is to mediate platelet adhesion, is the activated endothelium. ST2 is a member of the interleukin-1 receptor family. The soluble form of ST2 (sST2) may be secreted in response to stress or injury by a plethora of cell types, including immune cells, fibroblasts, and endothelial cells.[28] The levels of suPAR have been widely associated with markers of endothelial damage and activation, such as vWF, VCAM-1, and Syndecan-1.[29] The preliminary association of mEASIX with vWF, sST2, and suPAR seems to legitimate the capacity of the score to reflect endothelial activation.

Moreover, baseline mEASIX was able to predict the early development of DIC and CRS. These results highlight the role of a possible pre-existent endothelial impairment as a risk factor for those common toxicities.

Data on the predictive capacity of baseline mEASIX towards PFS and possibly on OS are in keeping with what was described by Pennisi and colleagues.[16] Modified EASIX is a combination of three parameters correlated to tumour burden, bone marrow function, and inflammatory status (LDH, platelet count, and CRP); as a consequence, endothelial dysfunction needs to be framed in the global condition of a high-risk patient. Variable association between mEASIX (with or without a cut-off) and OS probably depends on multiple scenarios occurring after progression, as some patients may receive palliative care, while others are treated with more intensive approaches, such as polatuzumab or tafasitamab-based regimens, or bispecific antibodies.

In agreement with previous reports, we have confirmed that consumptive coagulopathy appears as a laboratory finding more than a clinical syndrome, and tends to resolve, as long as the CRS is controlled.

Clinical manifestations of ICANS are associated with signs of endothelial damage, such as an increase in D-dimer, FVIII, and vWF antigen levels, in agreement with previous data.[11]

In conclusion, when CRS and ICANS develop after treatment with CAR-T cells, the majority of patients experience subclinical consumptive coagulopathy, which fulfils the ISTH criteria for diagnosis of DIC in one-third of cases. Coagulopathy is defined by increased PT and aPTT values, increased D-dimer levels, and decreased platelet count and antithrombin levels; consumption of fibrinogen is likely masked by a reactive increase due to the inflammatory setting. We also observed higher levels of FVIII and vWF antigen as signs of systemic inflammation and endothelial damage.

These laboratory alterations were associated with CRS grade 2–4 and with the mEASIX score for endothelial activation. Patients with impaired endothelium at day 0 may be at higher risk for developing CRS and overt DIC. Baseline mEASIX can also predict PFS.

Overall, the role of endothelium is emerging as pivotal in the pathophysiology of CRS and ICANS. Further studies are needed to explore the role of endothelial activation and coagulopathy in order to improve the safety and efficacy of CAR-T cell therapies.

AUTHOR CONTRIBUTIONS

Eugenio Galli, Federica Sorà, and Simona Sica designed the study and prepared the manuscript. Eugenio Galli, Federica Sorà, Alberto Fresa, Idanna Innocenti, Francesco Autore, Maria Assunta Limongiello, Sabrina Giammarco, Elisabetta Metafuni, Maria Assunta Limongiello, Luca Laurenti, Patrizia Chiusolo and Simona Sica managed the patients. Ilaria Pansini and Eugenio Galli collected and organized the data. Eugenio Galli performed the statistical analysis. Stefan Hohaus, Andrea Bacigalupo and Valerio De Stefano critically revised the draft of the paper, and gave important intellectual contribution. All authors critically reviewed and approved the paper.

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CONFLICT OF INTEREST

Eugenio Galli received honoraria for participating on advisory boards from Bristol Myers Squibb. Stefan Hohaus received honoraria for participating on advisory boards from Novartis. Patrizia Chiusolo received honoraria for participating on advisory boards from Bristol Myers Squibb and Novartis. Valerio De Stefano received honoraria for participating on advisory boards from Bristol Myers Squibb and Janssen. All other authors declare no competing conflicts of interests.

DATA AVAILABILITY STATEMENT

For data sharing, please contact the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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