CYTOKINE PROFILING FOR TRANSFUSION OUTCOME IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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ABSTRACT

Introduction:

In a previous clinical pilot study we demonstrated a significantly lower hemoglobin (Hb) increment in febrile patients receiving red blood cell (RBC) transfusions, compared to patients without fever. The aim of this study was to examine potential associations between inflammatory mediator profiles and transfusion outcome in in patients with hematological malignancies.

Methods:

Hb increment per unit transfused was corrected for estimated patient blood volume and the amount of Hb transfused. A wide spectrum of inflammatory mediators was determined by Luminex analysis. . We performed an unsupervised hierarchical cluster analysis to identify clusters of inflammatory mediators and investigate whether haematological patients with and without fever can be distinguished via such clusters. Association between transfusion outcomes, inflammation associated mediators in plasma samples and patient characteristics was analysed using a mixed linear regression model.

Results:

Patients with fever had significantly lower corrected Hb increment (Hb=0.48 g/dl) compared to patients without fever (Hb=0.71 g/dl) (p=0.002). In febrile patients four of the mediators demonstrated a significantly increased values (IL-6, IL-8, IL-10, and G-CSF) compared to non-febrile patients, whereas CCL2, CCL5 and CXCL10 levels in plasma were significantly lower. Febrile patients had a significantly lower corrected Hb increment compared to patients without fever, when adjusting for each of the 12 mediators. However, when temperature was kept constant, we found a significant negative association between transfusion outcome and the pro-inflammatory mediators IL-6 and CXCL-8.

Conclusion:

Febrile patients transfused with RBCs had significantly lower corrected Hb increment compared to patients without fever. Our results indicate that both fever and the inflammatory mediators IL-6 and CXCL-8 is negatively associated with transfusion outcome. Future studies are needed to further characterize this inflammatory response and its clinical implications.

INTRODUCTION

Anaemia occurs frequently in hospitalised cancer patients, decreases patients' quality of life (QOL) and may affect treatment outcome (1-3). In the "European Cancer Anaemia Survey"(ECAS) study, 39 % of cancer patients were anaemic at inclusion , and 67 % of the patients developed chemotherapy-induced cytopenia during a six month observation period (anaemia was defined as haemoglobin (Hb) < 12 g/dl) (4).

Treatment options for patients with chemotherapy induced anaemia include erythropoiesis stimulating agents and RBC transfusions (5). Platelet transfusion outcome is negatively affected by fever (6), and several pre-clinical studies indicate negative influence by fever on RBC transfusion outcome also. Experimentally induced fever caused increased Hb content in the spleen in rabbits, without any signs of intravascular hemolysis (7). RBCs stored for 35 days were more susceptible to fever than young red cells (8). Red cell response to fever is dependent on the degree of fever and the effects may be counteracted by administration of corticosteroids (9).

In a pilot study, we evaluated the Hb increment after RBC transfusions estimated by the difference between pre- and post- transfusion values corrected for the patient's estimated blood volume and amount haemoglobin transfused in haematologic patients with and without fever. The results indicate that haemoglobin increment per unit was significantly lower in febrile patients receiving RBC transfusions than in patients without fever (10).

The aim of the present study is to analyse inflammation-associated soluble mediators in plasma samples, and to investigate whether cytokine profiles in patients with and without fever receiving RBCs due to anaemia reflects a risk of transfusion outcome in patients with haematological malignancies.

MATERIALS AND METHODS

A prospecitve, observational study was performed.From June 2013 to July 2016 adult patients (age≥18) at the Section for Haematology, Department of Medicine, at Haukeland University Hospital with a hematologic disease requiring RBC transfusion were screened for inclusion.

Hemodynamically unstable patients (ongoing bleeding or hemolysis), or patients with a positive direct anti-globulin test (DAT) were excluded. The study period lasted until hospital discharge, although patients were eligible for re-inclusion if they were later admitted to hospital again. Indication for transfusion, Hb level (on the day of the transfusion), body temperature, height and weight were collected prior to transfusion. The patients and transfusion episodes were divided into two groups, "fever" and "no fever" (fever defined as a body temperature of ≥ 38 ^{o}C). Post transfusion hemoglobin concentration was determined in a blood sample taken either shortly after transfusion (15-60 minutes) or the following morning. Hb increment per unit transfused in the patient cohort was corrected for blood volume and the amount of Hb transfused, as previously described (10). The amount of Hb in the RBC units was calculated by a non-invasive method based on net weight of the concentrates as earlier published (11) and patients' blood volume was estimated by the DuBois and DuBois formula(12).

In accordance with hospital policy all patients received leucocyte depleted and irradiated RBCs.

RBC characteristics

The RBC concentrates (SAGM erythrocyte concentrates) were produced according to the standard procedure at the Department of Immunology and Transfusion Medicine at Haukeland University Hospital (Reveos system, Terumo BCT, Lakewood, CO, USA). All RBCs were leukocyte depleted by filtration before storage, and irradiated prior to transfusion. All were less than fourteen days old when they were irradiated, and maximum storage time after irradiation was until 28 days after collection. Prior to transfusion the following data were collected for the RBCs to be transfused; ABO and Rh (D) type, net weight and storage duration. The Hb content was determined based on the net weight of the concentrates as described earlier (11).

Preparation and preservation of plasma samples

Venous blood was drawn into citrate tubes (4 mL Vacuette Sodium Citrate tubes) by venipuncture. The blood samples were pipetted into Eppendorf tubes before they were centrifuged twice at 4°C for cytokine analysis; first at 2 500 G for 15 min. Plasma was then pipetted into Eppendorf tubes and centrifuged for 10 minutes at 10 000 G. The samples were aliquoted and immediately frozen at -70°C until analysed.

Multiplex technology

Mediator levels were determined by Luminex analysisusing the Bio-Plex Human Cytokine 27-plex kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). This kit includes the interleukins(IL) IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17 and interleukin 1 receptor antagonist (IL-1RA), the chemokines CCL-2 -3 -4 -5 -11 and CXCL-10, the growth factors basic fibroblast growth factor (FGF), platelet derived growth factor (PDGF-BB), granulocyte colony-stimulating factor (G-CSF), granulocyte

macrophage colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF) and the immunomodulatory cytokines interferon-γ (IFN-γ) and tumor necrosis factor-alpha (TNF-α). All analyses were performed strictly according to the manufacturer's instructions.

Ethics

The study was approved by the local Ethics Committee (Regional Ethics Committee III, University of Bergen, Norway), and samples were collected after written informed consent.

Statistical and bioinformatical analysis

A mixed model analysis (MMA) was used to account for repeated measures, and for 9 out of 27 patients being included more than once. P-values ≤ 0.05 were considered to be significant. The cytokine values were natural log (ln)-transformed before analysis to make the residuals more normally distributed. No serious deviation from normality of the residuals was found in the analyses.

A mixed linear regression model assuming a compound symmetry correlation structure between repeated measurements from the same patient was used to

1) compare characteristics of transfusion episodes between the two groups
2) compare the levels of white blood cells, neutrophil, granulocytes and CRP between the two groups
3) Compare the cytokine levels, adjusted for fever, between the two groups
4) Investigate if there was an association between each cytokine, adjusted for fever and corrected Hb increment.

Results are reported as estimated regression coefficients with 95 % confidence intervals and p-value from F-test.

Generalized estimating equations were used to test whether the proportion of patients transfused with platelet concentrates in addition to RBCs was independent of fever status, and to investigate whether unsupervised hierarchical clustering based on biologically related mediators could be used to identify patients with fever.

Bioinformatical analyses were performed using the J-Express (MolMine AS, Bergen, Norway) (13). For hierarchical clustering all values were median variance standardized and log (2) transformed. The complete linkage was used as linkage method, and for distance measured the Pearson correlation was used (13, 14). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software, CA) was used for the Figure presentation.

RESULTS

Patient characteristics

27 patients (8 female, 19 male) were included, none of the patients were splenectomized.15 patients were transfused only when they did not have fever, 8 patients had transfusion episodes both with and without fever, and 4 patients were transfused only when they had fever. A total number of 102 transfusion episodes, with 35 and 67 episodes in patients with and without fever, respectively, were evaluated.

Epidemiological data for the patient cohort are presented in **Table I**, and for the patients at each transfusion episode in **Table II**. The patients were diagnosed with a wide range of disorders; reflecting a consecutive and thereby unselected group of patients.

Pre- and post- transfusion Hb-level differed between patients with and without fever

Pre- and post-transfusion Hb- levels differed significantly between patients with and without fever (**Table II**). Pre-transfusion Hb level was significantly higher in patients with fever

compared to patients without fever (P=0.018), while post-transfusion Hb level was significantly lower in patients with fever (P=0.028). Consequently, patients with fever had significantly lower corrected Hb increment (mean corrected Hb increment 0.48 g/dl)) compared to patients without fever (mean corrected Hb increment 0.71 g/dl) (p=0.002) (**Figure I**).

Pre transfusion CRP levels differed significantly between patients with and without fever

We found that febrile patients had significantly higher pre–transfusion levels of CRP compared to patients without fever (P<0.001). Furthermore, we also found a significant association between pre-transfusion CRP levels and corrected Hb increment for the whole patient cohort. . However, when adjusting for fever there was no significant association between pre-transfusion CRP levels and corrected Hb increment within each group of patients. Neither the white blood cell (WBC) count nor the absolute neutrophil count (ANC) differed significantly between the patients with/without fever (**Table III**).

Platelet transfusion was significantly associated with fever

Febrile patients received both RBCs and platelet concentrate (PC) in 23/35 transfusion episodes, whereas patients without fever were transfused with PC in addition to RBCs in 28/67 transfusion episodes. We found a statistically significant association between fever and platelet transfusion (OR 2.9, P <0.001), indicating that there is 2.9 times increased probability that patients with fever are transfused with platelet concentrate compared to patients without fever.

Plasma levels of individual soluble mediators demonstrated variety in range within the cohort

Mediator levels were determined by Luminex analyses for 102 transfusion episodes from 27 different patients, 35 (34%) episodes in febrile patients and 67 (66%) episodes in patients without fever. The inflammatory mediators IL-1β, IL-2, IL-4, IL-5, IL-7, IL-12(p70), IL-13, IL-15, IL1-7A, basic FGF, PDGF-BB and VEGF showed undetectable or very low levels in all 102 samples (data not shown). Three mediators (IL-5, IL-7 and IL-9) demonstrated detectable levels in some samples, however low levels or undetectable levels for the majority of the samples (data not shown). In contrast, 12 mediators (TnF-α, IFN-γ, IL-1-rα, IL-6, IL-8, IL-10, CXCL-10, CCL-2, CCL-4, CCL-5, CCL-11 and G-CSF showed detectable levels in all the 102 samples analyzed. Therefore only these 12 latter mediators were used in further comparative statistical analysis.

For six of the mediators (IL- 6, IL-8, IL-10, CXCL-10, CCL5 and G-CSF), we detected statistically significant differences between the two groups. Four of the mediators (IL-6, IL-8, IL-10, and G-CSF) demonstrated a significantly higher values in febrile patients compared to non-febrile patients, whereas CCL5 and CXCL10 levels in plasma were significantly lower in patients with fever (**Figure I)**. These seven mediators represent a heterogeneous group with regard to biological function and include the pro-inflammatory mediators IL-6 and G-CSF, the anti-inflammatory mediators IL-1Rα and IL-10 and the pro-inflammatory chemokine IL-8(**Table IV, Figure I**).**Figure 1** shows absolute values of each mediator in patients with and without fever.

Unsupervised hierarchical clustering of 12 soluble mediators did not identify distinct cytokine clusters in patients with fever and corrected Hb increment less than mean value

To identify clusters of inflammatory mediators and investigate whether haematological patients with and without fever can be distinguished via such clusters, an unsupervised hierarchical cluster analysis of the inflammatory mediator profiles was performed.

We always included the first plasma sample, and only one plasma sample from each inclusion. Therefore, only 38 out of totally 102 samples in the study were included in the cluster analysis. As previously demonstrated by MMA, seven of the mediators (IL-6, IL-8, IL-10, CXCL 10, CCL2, CCL5 and G-CSF) were discriminative for patients with fever.

Figure II demonstrates unsupervised hierarchical clustering analysis of plasma levels for the 12 detectable inflammatory associated mediators,totally 38 plasma samples; (i) 10 samples from 9 patients (5 patients were included twice) with temperature >38 C^0, and 28 samples from 22 different patients (7 patients included twice, 1 patient included four times) with a temperature < 38 C^0 .

When clustering all 12 mediators, neither the febrile patients nor the plasma mediators did localize into certain clusters (**Figure II**). However, we found that some soluble mediators clustered together with or close to related mediators; i.e. cluster 1: IL 1Rα, TNF-α, IFN-γ, CCL2 and cluster 2: IL 6, IL8 and G-CSF (**Figure III**).To investigate whether unsupervised hierarchical clustering based on biologically related mediators could be used to identify patients with fever; we performed clustering analyses based on the seven mediators that differed significantly between febrile/non-febrile patients. In Cluster 1; 13/17 patients had fever, whereas in cluster 2: 3/13 patients had fever (Chi-Square test, p = 0.737). Thus, even when the cluster analysis was based only on those mediators showing statistically significant differences between patients with and without fever, the clustering cannot be used to separate patients with temperature +/- 38 C^0.

Association between transfusion outcomes, inflammations associated mediators in plasma samples and patient characteristics.

We performed analyses with body temperature as a dichotomous variable with the categories "Fever" and "Not fever", where fever was defined as a body temperature of 38^{o}C or more during the day (data not shown). 67 samples were analysed from patients without fever, and 35 samples from patients with fever. Tests of interaction (data not shown) revealed no significant interactions between haematological patients with and without fever, and the interaction variable was therefore omitted from further analysis. The results of the linear mixed model analysis of the impact of the patients characteristics (data not shown); age, gender and diagnosis, revealed no significant association between patient characteristics and transfusion outcome, and was also omitted from further statistical analysis.

First, we tested if there was an association between each of the mediators and corrected Hb increment when adjusting for fever (dichotomous variable, fever and no fever, fever defined as a body temperature $\geq$ 38 ^{o}C). Mixed model analysis was used to account for repeated measures and for some of the patients being included more than once. Corrected Hb increment was outcome variable and each mediator and fever was predictor variables. The mediators were ln-transformed to make the residuals more normally distributed in order to meet the assumptions of the statistical model. "Estimate intercept" is the estimated value for the intercept of the curve for the "Fever" group when ln(mediator) is zero. "Estimate No fever" is the difference in corrected Hb increment between the "Fever" and the "No fever" group when the mediator level is kept constant. "Estimate Mediator" is the change in corrected Hb increment as ln(mediator) increases with 1 and fever is kept constant. The estimates are given with 95% confidence intervals, and "Estimate No fever" and "Estimate Mediator" also have p-values reported.

The results of the MLA demonstrated that haematological patients with fever had a significantly lower corrected Hb increment compared to patients without fever, when adjusting for each of the 12 mediators (**Table V**). However, when temperature was kept constant, we found a significant negative association between transfusion outcome and the pro-inflammatory mediators IL-6($p<0.001$) and CXCL-8($p<0.001$)I,indicating that when fever is kept constant an increase in IL-6 and CXCL-8 results in a decrease in corrected Hb increment equivalent to regression coefficient.

DISCUSSION

In a pilot study, we evaluated Hb increment after RBCs transfusion by calculating the difference between pre- and post- transfusion values corrected for the patients blood volume and gram hemoglobin transfused in hematologic patients with and without fever. The results indicate that hemoglobin increment per unit was significantly lower in febrile patients receiving red cell transfusions than in patients without fever (10). The aims of the present study were to analyze twenty-seven inflammation-associated soluble mediators in plasma samples, and to investigate whether mediator profiles is associated with transfusion outcome. Although our patient group is relatively small, we would emphasize that all samples were harvested consecutively from patients with hematological malignancies. Thus our patient cohort represents consecutive and unselected patients with hematological malignancies.

Fever in our patient cohort could be triggered for various reasons including cancer, infections and other causes such as drug fever, thrombotic thrombocytopenic purpura and deep venous thrombosis (referanse). Inflammatory mediators involved in induction of the inflammatory response are the pro-inflammatory cytokines TNF-α, IL-1, IL-6 and IL-8 and the anti-inflammatory cytokines IL-10 and IL- 1RA. Fever is mediated by the release of pyrogenic mediators such as TNF-α, IL-1, IL-6, and interferons into the bloodstream in response to exogenous pyrogens. In our study we found upregulated pre-transfusion levels of IL- 6 in hematological patients with fever compared to patients without fever; however we found no significant differences for TNF-α. Our results demonstrate that IL- 6 was part of a broader inflammatory response which may be associated with a risk of transfusion outcome in patients with hematological malignancies.

C-reactive protein (CRP), an acute phase protein, is mainly synthesized by the hepatocytes in the liver in response to IL-6(15). Previous studies have demonstrated a strong

correlation between CRP and IL-6 levels (16).Elevated levels of serum CRP is observed with most invasive infections(17). In addition, several other conditions contribute to elevated CRP concentrations including; trauma, surgery, burns, tissue necrosis, immunologically mediated inflammatory diseases, crystal-induced inflammatory diseases and advanced cancer (15, 18).

We hypothesized that there exists a relationship between pre-transfusion CRP level in febrile and non-febrile hematological patients and transfusion outcome, and the level of inflammation, as measured by CRP. Our results demonstrated that febrile patients had significantly higher pre –transfusion CRP levels compared to patients without fever. We also found a significant association between pre-transfusion CRP levels and corrected delta Hb increment, indicating that increase in CRP level equal to one unit, results in a decrease in corrected delta HB equivalent to the regression coefficient. However, when we kept temperature constant at a certain value we found no significant association between pre transfusion CRP levels and corrected delta Hb, indicating that changes in temperature and not changes in pre transfusion CRP levels explains changes in corrected delta HB.

Platelet concentrates contain soluble mediators derived from both platelets and contaminating leukocytes. During platelet transfusion these mediators are transferred to the recipient. In a previous study by Apelseth(ref), they investigated the effect of platelet transfusion on the systemic levels of inflammatory mediators in acute leukemia patients with severe chemotherapy-induced cytopenia(referanse). Their results demonstrated altered levels of a number of soluble mediators, including a transient decreased level of IL-6 and increased plasma levels of CCL5. Our results demonstrated a statistically significant association between fever in the patient cohort and platelet transfusion (OR 2.9, P <0.001), indicating that there is 2.9 times increased probability that patients with fever are transfused with platelet concentrate compared to patients without fever. When comparing pre-transfusion levels of mediators between febrile/non-febrile patients we found decreased levels of IL-6 and increased level of CCL, indicating that platelet transfusions modulate the systemic cytokine network in acute leukemia patients with chemotherapy-induced cytopenia.

When comparing pre –transfusion levels of mediators in febrile/non- febrile patients, we identified significantly higher level of the pro-inflammatory cytokines IL-6, IL-8, the anti-inflammatory cytokine IL10 and growth factor G-CSF, whereas we found reduced levels of the chemokines CCL2, CCL5 and CXCL10 in patients with fever. Our results demonstrated a variety in range of inflammatory mediators. Previous studies have demonstrated elevated levels of TNF- α and IL-6 in patients with chemotherapy-induced cytopenia compared to healthy controls. Our study compared pre-transfusion levels of mediators in hematological patients with chemotherapy-induced cytopenia +/- fever did not include healthy controls(ref). The results demonstrated altered levels of 7 inflammatory mediators. Thus, intensive chemotherapy does not cause a general depression of serum cytokine levels.

We used an unsupervised hierarchical clustering method to obtain a more complementary picture in our heterogeneous data set. In our study, this methodology was used to explore inflammatory mediators as well as patient covariates, as it gives an overview of both patient as well as covariates in one single picture that is difficult to obtain using traditional statistics.

We included total number 38 samples collected from 27 patients in the analysis. However, when performing usupervised hierarchical clustering analysis our results did not demonstrate statistically significant differences between patients with and without fever, which indicate that the clustering cannot be used to separate patients with temperature +/- 38 C^0.

We looked for associations between transfusion outcomes; inflammations associated mediators in plasma samples and patient characteristics (age, gender and diagnosis). Neither, gender, age or diagnosis did demonstrated any significant association to transfusion outcome and were also omitted from the analysis, as shown Table ? . In the first analysis, our results demonstrated that when each of the mediators was kept constant for a certain value, febrile patients had a significantly lower corrected Hb increment compared to hematological patients

without fever. However, when temperature was kept constant, only the cytokine IL-6 and chemokine CCL-8 demonstrated a significant negative correlation between transfusion outcome and corrected Hb increment in patients with fever. These results indicate; (i) when the p- value for Fever <0.05 and the and the p-value for the mediator >0.05, fever contributes to explain the lower Hb increment in febrile patients compared to non-febrile haematological patients, (ii) when the p-value for fever <0.05 and the p-value for the mediator <0.05 both fever and mediator contributes independently of each other to explain the lower Hb increment in febrile patients.

Weaknesses:

In our study there was no fixed time of measuring the post-transfusion Hb increment, and median time is therefor unfortunately not known. However, 26 samples were collected shortly after the end of transfusion and 103 samples the following morning. The mean haemoglobin value from the 26 samples collected shortly after transfusion were 0.1 g/dl less than the mean haemoglobin value the following morning and the difference is not statistically significant($p = 0.755$)

CONCLUSION

In conclusion, hematological patients with fever transfused with RBCs had significantly lower corrected Hb increment compared to patients without fever. Our results indicate that both fever and the inflammatory mediators IL-6 and CXCL-8 is associated with transfusion outcome. Future studies are needed to further characterize this inflammatory response and its clinical implications.

REFERENCES

1. Demetri GD. Anaemia and its functional consequences in cancer patients: current challenges in management and prospects for improving therapy. British journal of cancer. 2001;84 Suppl 1:31-7.
2. Ludwig H, Strasser K. Symptomatology of anemia. Seminars in oncology. 2001;28(2 Suppl 8):7-14.
3. Glaspy J. Anemia and fatigue in cancer patients. Cancer. 2001;92(6 Suppl):1719-24.
4. Ludwig H, Van Belle S, Barrett-Lee P, Birgegard G, Bokemeyer C, Gascon P, et al. The European Cancer Anaemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. Eur J Cancer. 2004/09/30 ed2004. p. 2293-306.
5. Scialdone L. Overview of supportive care in patients receiving chemotherapy: antiemetics, pain management, anemia, and neutropenia. J Pharm Pract. 2012;25(2):209-21.
6. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood. 2005;105(10):4106-14.
7. Karle H. The site of abnormal erythrocyte destruction during experimental fever. Br J Haematol. 1968;15(5):475-85.
8. Karle H. Significance of red cell age to red cell destruction during experimental pyrexia. Br J Haematol. 1968;15(3):221-9.
9. Karle H. Elevated body temperature and the survival of red blood cells. A study on experimental pyrexia in rabbits. Acta Med Scand. 1968;183(6):587-92.
10. Wendelbo O, Netland Opheim E, Felli Lunde TH, Bruserud O, Hervig T, Reikvam H. A prospective observational study on effects of fever on red cell transfusion outcome. Vox Sang. 2017;112(5):484-6.
11. Reikvam H, van de Watering L, Prowse C, Devine D, Heddle NM, Hervig T. Evaluation of noninvasive methods for the estimation of haemoglobin content in red blood cell concentrates. Transfusion medicine (Oxford, England). 2011;21(3):145-9.
12. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition. 1989;5(5):303-11; discussion 12-3.
13. Stavrum AK, Petersen K, Jonassen I, Dysvik B. Analysis of gene-expression data using J-Express. Curr Protoc Bioinformatics. 2008;Chapter 7:Unit 7.3.
14. Reikvam H, Mosevoll KA, Melve GK, Gunther CC, Sjo M, Bentsen PT, et al. The pretransplantation serum cytokine profile in allogeneic stem cell recipients differs from healthy individuals, and various profiles are associated with different risks of posttransplantation complications. Biol Blood Marrow Transplant. 2012;18(2):190-9.
15. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. The New England journal of medicine. 1999;340(6):448-54.

16. Oberhoffer M, Karzai W, Meier-Hellmann A, Bogel D, Fassbinder J, Reinhart K. Sensitivity and specificity of various markers of inflammation for the prediction of tumor necrosis factor-alpha and interleukin-6 in patients with sepsis. Critical care medicine. 1999;27(9):1814-8.
17. Jaye DL, Waites KB. Clinical applications of C-reactive protein in pediatrics. The Pediatric infectious disease journal. 1997;16(8):735-46; quiz 46-7.
18. Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. The Journal of clinical investigation. 1993;91(4):1351-7.

Table I. Epidemiologic data for the 27 patients included in the study

Patient demographics	
Age (median (min-max)):	56 (20-71)
Male (n (%)):	19 (70.4)
Height in cm (median (min-max))	178 (157-197)
Diagnosis (n (%))	
Myelodysplastic syndrome/acute myelogenous leukemia (MDS/AML)	18 (66.6)
Acute lymphomblastic leukemia (ALL)	2 (7.4)
Chronic lymphatic leukemia (CLL)	1 (3.7)
Acute promyelocytic leukemia (APL)	1 (3.7)
Multiple myeloma (MM)	1 (3.7)
Histiocytic sarcoma (HS	1 (3.7)
Myelofibrosis (MF)	1 (3.7)
Mantle cell lymphoma (MCL)	1 (3.7)
Unspecified anaemia	1 (3.7)

Table II. Epidemiological data for the 27 patients and the 102 transfusion episodes in patients with hemato
are given as mean, standard deviation (SD) and minimum-maximum.

	No fever (mean(SD)
Transfusion episodes	67
Weight(kilogram)	79.39 (2.95)(49.4, 11
BSA(m^2)	1.954 (0.037)(1.4
EBV(liter)	5.093 (0.143)(3.1
Number of units transfused in each episode	2.0 (0.48)(1,
Hemoglobin transfused(gram)	103.39 (2.70)(48.2
Pre-transfusion Hb(gram/dl)	7.98 (0.08)(6.2
Post-transfusion HB(gram/dl)	9.46 (0.10)(8.0,
Hb increment/unit (g/dl)	0.73 (0.04)(0.0
Hemoglobin transfused /EBV(gram/liter)	21.153(0.828)(9.0
Hb increment adjusted for blood volume and hb transfused	0.707 (0.040)(0.0

Table III: Characteristics of pre-transfusion biochemistry in the study population. Due to repeated analysis we used a
patients being included more than once.

	No fever(mean(St. error)(min-max)) n=67
WBC* ($\times 10^9$/L)	2.70(1.05)(0.10-37.5)
ANC** (x 10^9/L)	1.76(0.82)(0.00-24.6)
CRP level (mg/L)	59.6(8.37)(3-297)
Platelet concentrate transfused***	28/67

* White Blood Cell
** Absolute Neutrophil Count

***Generalized Estimating Equation

Table 4

Mediator levels were measured in 102 samples from 27 patients with hematological malignancies with and without fever. Sixty-seven samples from patients with a temperature < 38 ^{0} C, and 35 samples from patients with temperature > 38^{0} C. Mixed model analysis (MMA) was performed with each mediator as outcome and fever as predictor. Mean and p-values from MMA. All values measured in picogram/milliliter (pg/ml)

Figure I. Significant differences in plasma levels of soluble inflammatory mediators; a comparison of hematogical patients with fever and without fever. The figure presents the results for inflammatory mediators showing statistically significant differences. The p-values are calculated by Mixed Linear Model analysis to account for repeated measures, and for patients being included more than once, and are indicated in the figure. The mean levels are marked as horizontal lines. All values are given as pg/ml.

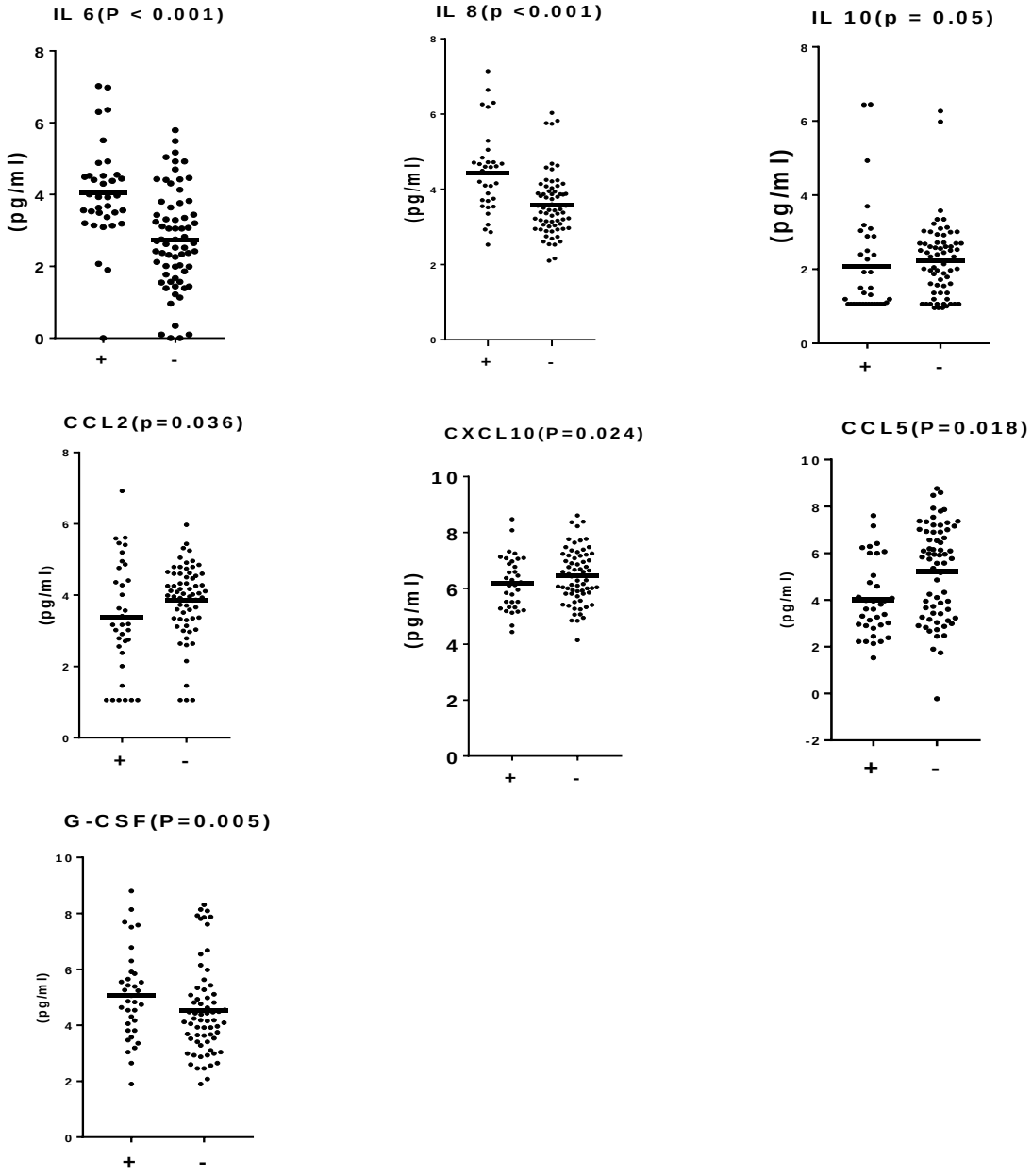

Figure II. Unsupervised hierarchical clustering analysis of plasma levels for the 12 detectable inflammatory associated mediators including 38 samples from 27 patients. To account for repeated measures, and for patients being included more than once, the cluster analysis was always based only on the first sample from each admission. In the heat map the concentrations were median normalized and log (2) converted before Euclidian correlation test with complete linkage were for the clustering analysis. The mediators are clustered

horizontally, while the patients are clustered vertically. The final diagnosis for each patient is given in the right part of the figure.

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Figure III. Unsupervised hierarchical clustering analysis of plasma levels for the 7 biomarkers that demonstrated significant differences when comparing mediator levels from febrile/non febrile patients. The cluster analysis included 38 samples from 27 patients. To account for repeated measures, and for patients being included more than once, the analysis was always based only on the first sample from each admission. The plasma concentrations were median normalized and log (2) converted before Euclidian correlation test with complete linkage was used for the clustering analysis. The mediators are clustered horizontally, while the patients are clustered vertically. The final diagnosis for each patient is indicated in the right part of the figure.

Figure V: Association between transfusion outcomes, inflammations associated mediators in plasma samples and patient characteristics. A mixed linear regression model was use to relate each cytokine to the patient characteristics assuming a compound symmetry correlation structure between repeated measurements from the same patient at different stays. Results are reported as estimated regression coefficients with 95 % confidence intervals and p-value from F-test. The cytokine values were natural log (ln)-transformed before analysis to fulfill the assumption of normally distributed response.

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