Inflammatory molecule reduction with hydroxyurea therapy in children with sickle cell anemia

Rhiannon R. Penkert,[1] Julia L. Hurwitz,[1,2] Paul Thomas,[3] Jason Rosch,[1] Jola Dowdy,[4] Yilun Sun,[5] Li Tang[5] and Jane S. Hankins[4]

Departments of [1]Infectious Diseases, [3]Immunology, [4]Hematology, and [5]Biostatistics, St. Jude Children's Research Hospital and [2]Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA

Correspondence: jane.hankins@stjude.org
doi:10.3324/haematol.2017.177360

Supplemental Materials for:

Inflammatory molecule reduction with hydroxyurea therapy in children with sickle cell anemia

Rhiannon R. Penkert[1]; Julia L. Hurwitz[1,2]; Paul Thomas[3]; Jason Rosch[1]; Jola Dowdy[4]; Yilun Sun[5]; Li Tang[5]; and Jane S. Hankins[4]

Departments of [1]Infectious Diseases, [3]Immunology, [4]Hematology, and [5] Biostatistics, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105 USA; [2]Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN 38163 USA.

MATERIALS AND METHODS

Participant selection

Children with sickle cell disease of all genotypes, ranging in age from 5.0 to 18.9 years, were prospectively enrolled in a longitudinal study investigating echocardiographic measurements and laboratory markers of inflammation, coagulation and hemolysis (NCT 00842621). All were followed by St. Jude Children's Research Hospital, pediatric Sickle Cell Disease program, Memphis, TN. In the current report, we present the inflammatory molecule assessments of HbSS and HbSβ^0-thalassemia (SCA) study participants. All subjects were sampled at least once during the study ('baseline') for evaluation of differences among treatment groups. A subset was sampled longitudinally after a 2-year observation period ('follow-up'), for analysis of the effects of treatment over time. At baseline, patients with SCA were sub-classified according to their treatment status: (i) those not receiving any form of disease-modifying therapy (untreated), (ii) those receiving hydroxyurea therapy at the maximum tolerated dose (MTD) (i.e., after completion of dose escalation to maximum dose

defined by mild myelossupression) (1, 2), and (iii) those receiving chronic blood transfusion treatment for at least 6 months. Patients received erythrocyte transfusions once a month. Patients who had not completed the hydroxyurea dose-escalation period (typically the initial 6 months of treatment) and patients who had received monthly chronic blood transfusions for less than six months were not included in evaluations, to avoid possible variability introduced by an incomplete treatment effect. For the longitudinal analyses, patients were grouped as follows: (i) those who did not receive any disease-modifying treatment throughout the 2-year observation period, (ii) those who remained on hydroxyurea treatment continuously, (iii) those who remained on chronic transfusions continuously, and (iv) those who were initially untreated and initiated therapy with hydroxyurea during the study period (Supplementary Table 1). The median age of patients at baseline was 10.4 years (range 5.2 to 18.8 years); 105 patients were male; 109 patients were female; all patients were African American. The SCA subgroups, according to treatment status at baseline assessment, were as follows: untreated (N=118, median age 8.6, range 5.2 to 18.8 years), treated with hydroxyurea (N=72, median age 13.3, range 5.3 to 18.1 years), or treated with chronic monthly blood transfusions (N=24, median age 13.4, range 6.0 to 18.4 years). Indications for hydroxyurea initiation included recurrent vaso-occlusive events (VOE), chronic hypoxemia, Hb <7 g/dl, fetal Hb level <8% after 24 months of age, white blood cell count $>20 \times 10^9$/L, or lactate dehydrogenase > twice the upper limit of normal (3). Indications for chronic monthly blood transfusion initiation included primary prevention of stroke due to abnormal transcranial doppler velocities (4), or secondary stroke prevention (5). At baseline assessment, the median duration of hydroxyurea treatment was 4.7 years (range, 0.7 to 15.7 years), at a median dose of 25.5 mg/kg/day (range, 15.9-31.3 mg/kg/day). At baseline, the median duration of transfusion therapy among chronically transfused children was 6.3 years (range, 0.5 to 15.0 years).

In longitudinal, paired analyses, twenty-six children who were untreated at the time of baseline sampling and initiated hydroxyurea at least 6 months prior to the time of their 2-year sampling were studied ("initiating hydroxyurea"). The median age of this group at the time of baseline sampling was 11.1 years (range 5.7 to 16.8

years), and the median duration of treatment at the time of the 2-year sampling was 1.3 years (range, 54 days to 2 years), at a median dose of 21 mg/kg/day (range, 14-30 mg/kg/day). Additionally, forty-one children who were on hydroxyurea at the time of baseline sampling and remained on hydroxyurea therapy throughout the 2-year observation period ("continuous hydroxyurea") were evaluated for the same inflammatory markers at both baseline and the 2-year assessment date. Their duration of therapy at the time of baseline sampling was 4.8 years (0.9 to 15.2 years), at a median dose of 26 mg/kg/day (range, 18-31 mg/kg/day). Seventeen participants receiving transfusions at the time of baseline sampling remained on this therapy and were re-evaluated at the end of the 2-year observational period (median age 12.1 years, range 6.0 to 17.6 years). Thirty-eight children remained untreated throughout the 2-year interval [median age of 8.3 years (range 5.5 to 16.4 years)].

Controls evaluated in this study were residual samples from uninfected healthy asymptomatic children who shared a household with an influenza virus-infected person (Flu09 study, (6)). The St. Jude institutional review board approved the study and all legal guardians or participants (if at age of majority during the follow-up interval) provided written informed consent prior to any study-related activity.

Evaluations

Blood samples were collected during steady-state (i.e., no fever, pain or any other acute disease complication for >30 days). A sample for clinical inflammatory markers (white blood cell counts, platelet count, Von Willebrand antigen, C-Reactive protein, D-dimer, and Factor VIII antigen level), and another sample for inflammatory molecules (cytokines, chemokines, and adhesion molecules) was obtained. Samples for inflammatory molecules were spun twice at 3000 rpm in a Sorvall, Legend RT centrifuge for 10 min within 30 minutes of collection, and subsequently frozen at -85°C. Frozen serum samples were thawed and centrifuged briefly (1000g for 5 minutes) to pellet any debris, and then tested for the presence of 41 different cytokines, chemokines and adhesion molecules using the following kits: 1) Human Cytokine/Chemokine Magnetic Bead Panel Milliplex MAP Kit (Millipore; cat. no. HCYTOMAG-60K-PX41), 2) Human Cardiovascular Disease

(CVD) Panel 2 Magnetic Bead Milliplex MAP Kit (Millipore; cat. no. HCVD2MAG-67K), and 3) Human sE-selectin ELISA kit (Invitrogen; cat. no. KHS2011). Cytokines, chemokines and adhesion molecules examined included: sCD40L, EGF, Eotaxin, E-selectin, FGF-2, FKN, Flt-3L, G-CSF, GM-CSF, GRO, sICAM-1, IFNα2, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL12(p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, TGFα, TNFα, TNFβ, sVCAM-1, VEGF. Inflammatory molecules were selected for testing due to clinical significance and commercial availability of comprehensive premixed kits. Samples, controls, and standards were prepared and processed per manufacturer's instructions. Samples were used undiluted in the Cytokine/Chemokine Panel and diluted 1:100 in assay buffer prior to use in the Human CVD Panel. All Milliplex assays were read on a Luminex 200 Multiplexing Instrument using xPonent software and data were processed using Milliplex Analyst software. It should be noted that while Millipore rigorously tests and ensures the precision (<20% CV inter-assay) and accuracy (87-105% recovery in serum) of their multiplex assays, it has been reported in the literature that when multiplexed, a small number of the analytes evaluated in our study may lack detectability (<25%) or reproducibility (>20% CV between assays), including IL-4, IL7, IL-12(p70) and TGFα (7). The E-selectin ELISA was performed according to the Invitrogen handbook, and read on a Molecular Devices Precision Microplate Reader. Samples were diluted 1:5 in sample diluent prior to use in the ELISA.

Statistical considerations

Baseline inflammatory molecule levels were compared between patients with SCA and healthy controls using the Mann Whitney U test, however when >75% of levels were below the limit of detection (LOD), the Fisher's exact test was used instead. For inflammatory molecules with at least 25% measurements within the detection limits, Kruskal-Wallis test followed by a Dunns pairwise test was performed to evaluate statistically significant differences among patients groups (SCA patients treated with hydroxyurea, SCA patients treated with chronic blood transfusions, untreated SCA patients, and healthy controls). To confirm the significance, log transformed values of inflammatory molecules were also compared with Welch's analysis of variance. P-values were

adjusted for multiple testing across inflammatory molecules with the Benjamini-Hochberg false-discovery rate method (8). Any change of inflammatory molecule levels from baseline to follow-up was analyzed using the Wilcoxon signed rank test. The Spearman's rank correlation coefficient was calculated to assess the correlation of inflammatory molecule levels with clinical inflammatory markers. Inflammatory molecule levels below or above the LOD were reported as the LOD. VOEs that led to hospitalizations were collected for the 2 years prior to baseline and during the 2 year of observation and their incidence rates compared using the paired t-test. Data analyses were performed using Excel, GraphPad Prism (v5) and SAS 9.4 (SAS Institute).

Supplemental References

1. Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group. Blood. 1999;94(5):1550-1554.

2. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood. 2010;115(26):5300-5311.

3. Heeney MM, Ware RE. Hydroxyurea for children with sickle cell disease. Hematol Oncol Clin North Am. 2010;24(1):199-214.

4. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998;339(1):5-11.

5. DeBaun MR, Kirkham FJ. Central nervous system complications and management in sickle cell disease. Blood. 2016;127(7):829-838.

6. Oshansky CM, Gartland AJ, Wong SS, et al. Mucosal immune responses predict clinical outcomes during influenza infection independently of age and viral load. AmJ RespirCrit Care Med. 2014;189(4):449-462.

7. Chaturvedi AK, Kemp TJ, Pfeiffer RM, et al. Evaluation of multiplexed cytokine and inflammation marker measurements: a methodologic study. Cancer Epidemiol Biomarkers Prev. 2011;20(9):1902-1911.

8. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J Roy Stat Soc B Met. 1995;57(1):289-300.

9. Garrido VT, Sonzogni L, Mtatiro SN, et al. Association of plasma CD40L with acute chest syndrome in sickle cell anemia. Cytokine. 2017;97:104-107.

10. Aloui C, Prigent A, Tariket S, et al. Levels of human platelet-derived soluble CD40 ligand depend on haplotypes of CD40LG-CD40-ITGA2. Sci Rep. 2016;6:24715.

11. Lee SP, Ataga KI, Orringer EP, et al. Biologically active CD40 ligand is elevated in sickle cell anemia: potential role for platelet-mediated inflammation. Arterioscler Thromb Vasc Biol. 2006;26(7):1626-1631.

12. Davila J, Manwani D, Vasovic L, et al. A novel inflammatory role for platelets in sickle cell disease. Platelets. 2015;26(8):726-729.

13. Matthies KM, Newman JL, Hodzic A, Wingett DG. Differential regulation of soluble and membrane CD40L proteins in T cells. Cell Immunol. 2006;241(1):47-58.

14. Kasza A. IL-1 and EGF regulate expression of genes important in inflammation and cancer. Cytokine. 2013;62(1):22-33.

15. Berrahmoune H, Lamont JV, Herbeth B, et al. Biological determinants of and reference values for plasma interleukin-8, monocyte chemoattractant protein-1, epidermal growth factor, and vascular endothelial growth factor: Results from the STANISLAS cohort. Clin Chem. 2006;52(3):504-510.

16. Adar T, Shteingart S, Ben Ya'acov A, et al. From airway inflammation to inflammatory bowel disease: eotaxin-1, a key regulator of intestinal inflammation. Clin Immunol. 2014;153(1):199-208.

17. Rankin SM, Conroy DM, Williams TJ. Eotaxin and eosinophil recruitment: implications for human disease. Mol Med Today. 2000;6(1):20-27.

18. Lebensburger JD, Howard T, Hu Y, et al. Hydroxyurea therapy of a murine model of sickle cell anemia inhibits the progression of pneumococcal disease by down-modulating E-selectin. Blood. 2012;119(8):1915-1921.

19. Telen MJ. Cellular adhesion and the endothelium: E-selectin, L-selectin, and pan-selectin inhibitors. Hematol Oncol Clin North Am. 2014;28(2):341-354.

20. Pathare A, Kindi SA, Daar S, Dennison D. Cytokines in sickle cell disease. Hematology. 2003;8(5):329-337.

21. Bochner BS, Sterbinsky SA, Bickel CA, et al. Differences between human eosinophils and neutrophils in the function and expression of sialic acid-containing counterligands for E-selectin. J Immunol. 1994;152(2):774-782.

22. Yu PJ, Ferrari G, Galloway AC, et al. Basic fibroblast growth factor (FGF-2): the high molecular weight forms come of age. J Cell Biochem. 2007;100(5):1100-1108.

23. Allouche M, Bikfalvi A. The role of fibroblast growth factor-2 (FGF-2) in hematopoiesis. Prog Growth Factor Res. 1995;6(1):35-48.

24. Bazan JF, Bacon KB, Hardiman G, et al. A new class of membrane-bound chemokine with a CX3C motif. Nature. 1997;385(6617):640-644.

25. Clark AK, Staniland AA, Malcangio M. Fractalkine/CX3CR1 signalling in chronic pain and inflammation. Curr Pharm Biotechnol. 2011;12(10):1707-1714.

26. Jones BA, Beamer M, Ahmed S. Fractalkine/CX3CL1: a potential new target for inflammatory diseases. Mol Interv. 2010;10(5):263-270.

27. Geiser T, Dewald B, Ehrengruber MU, et al. The interleukin-8-related chemotactic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. J Biol Chem. 1993;268(21):15419-15424.

28. Conran N, Fattori A, Saad ST, Costa FF. Increased levels of soluble ICAM-1 in the plasma of sickle cell patients are reversed by hydroxyurea. Am J Hematol. 2004;76(4):343-347.

29. Duits AJ, Rodriguez T, Schnog JJ, Group CS. Serum levels of angiogenic factors indicate a pro-angiogenic state in adults with sickle cell disease. Br J Haematol. 2006;134(1):116-119.

30. Okpala I. Leukocyte adhesion and the pathophysiology of sickle cell disease. Curr Opin Hematol. 2006;13(1):40-44.

31. Marlin SD, Staunton DE, Springer TA, et al. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. Nature. 1990;344(6261):70-72.

32. Witkowska AM, Borawska MH. Soluble intercellular adhesion molecule-1 (sICAM-1): an overview. Eur Cytokine Netw. 2004;15(2):91-98.

33. Brierley MM, Fish EN. Review: IFN-alpha/beta receptor interactions to biologic outcomes: understanding the circuitry. J Interferon Cytokine Res. 2002;22(8):835-845.

34. Paul F, Pellegrini S, Uze G. IFNA2: The prototypic human alpha interferon. Gene. 2015;567(2):132-137.

35. Wang T, Secombes CJ. The evolution of IL-4 and IL-13 and their receptor subunits. Cytokine. 2015;75(1):8-13.

36. Katzman SD, Hoyer KK, Dooms H, et al. Opposing functions of IL-2 and IL-7 in the regulation of immune responses. Cytokine. 2011;56(1):116-121.

37. Sun L, He C, Nair L, Yeung J, Egwuagu CE. Interleukin 12 (IL-12) family cytokines: Role in immune pathogenesis and treatment of CNS autoimmune disease. Cytokine. 2015;75(2):249-255.

38. Belperio JA, Keane MP, Arenberg DA, et al. CXC chemokines in angiogenesis. J Leukoc Biol. 2000;68(1):1-8.

39. Dufour JH, Dziejman M, Liu MT, et al. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol. 2002;168(7):3195-3204.

40. Laurance S, Pellay FX, Dossou-Yovo OP, et al. Hydroxycarbamide stimulates the production of proinflammatory cytokines by endothelial cells: relevance to sickle cell disease. Pharmacogenet Genomics. 2010;20(4):257-268.

41. Mantovani A, Gray PA, Van Damme J, Sozzani S. Macrophage-derived chemokine (MDC). J Leukoc Biol. 2000;68(3):400-404.

42. Vulcano M, Albanesi C, Stoppacciaro A, et al. Dendritic cells as a major source of macrophage-derived chemokine/CCL22 in vitro and in vivo. Eur J Immunol. 2001;31(3):812-822.

43. Cook DN. The role of MIP-1 alpha in inflammation and hematopoiesis. J Leukoc Biol. 1996;59(1):61-66.

44. Baba T, Mukaida N. Role of macrophage inflammatory protein (MIP)-1alpha/CCL3 in leukemogenesis. Mol Cell Oncol. 2014;1(1):e29899.

45. Maurer M, von Stebut E. Macrophage inflammatory protein-1. Int J Biochem Cell Biol. 2004;36(10):1882-1886.

46. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 2002;13(6):455-481.

47. Partridge M, Green MR, Langdon JD, Feldmann M. Production of TGF-alpha and TGF-beta by cultured keratinocytes, skin and oral squamous cell carcinomas--potential autocrine regulation of normal and malignant epithelial cell proliferation. Br J Cancer. 1989;60(4):542-548.

48. Groenen LC, Nice EC, Burgess AW. Structure-function relationships for the EGF/TGF-alpha family of mitogens. Growth Factors. 1994;11(4):235-257.

49. Sarray S, Saleh LR, Lisa Saldanha F, et al. Serum IL-6, IL-10, and TNFalpha levels in pediatric sickle cell disease patients during vasoocclusive crisis and steady state condition. Cytokine. 2015;72(1):43-47.

50. Vilas-Boas W, Veloso Cerqueira BA, Figueiredo CV, et al. Association of homocysteine and inflammatory-related molecules in sickle cell anemia. Hematology. 2016;21(2):126-131.

51. Keikhaei B, Mohseni AR, Norouzirad R, et al. Altered levels of pro-inflammatory cytokines in sickle cell disease patients during vaso-occlusive crises and the steady state condition. Eur Cytokine Netw. 2013;24(1):45-52.

52. Lanaro C, Franco-Penteado CF, Albuqueque DM, et al. Altered levels of cytokines and inflammatory mediators in plasma and leukocytes of sickle cell anemia patients and effects of hydroxyurea therapy. J Leukoc Biol. 2009;85(2):235-242.

53. Pfeffer K. Biological functions of tumor necrosis factor cytokines and their receptors. Cytokine Growth Factor Rev. 2003;14(3-4):185-191.

54. Alevriadou BR. CAMs and Rho small GTPases: gatekeepers for leukocyte transendothelial migration. Focus on "VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration". Am J Physiol Cell Physiol. 2003;285(2):C250-252.

55. Dworkis DA, Klings ES, Solovieff N, et al. Severe sickle cell anemia is associated with increased plasma levels of TNF-R1 and VCAM-1. Am J Hematol. 2011;86(2):220-223.

56. Lancelot M, White J, Sarnaik S, Hines P. Low molecular weight heparin inhibits sickle erythrocyte adhesion to VCAM-1 through VLA-4 blockade in a standardized microfluidic flow adhesion assay. Br J Haematol. 2017;178(3):479-481.

57. Makis AC, Hatzimichael EC, Stebbing J, Bourantas KL. C-reactive protein and vascular cell adhesion molecule-1 as markers of severity in sickle cell disease. Arch Intern Med. 2006;166(3):366-368.

58. Keramaris NC, Calori GM, Nikolaou VS, et al. Fracture vascularity and bone healing: a systematic review of the role of VEGF. Injury. 2008;39 Suppl 2:S45-57.

59. Al-Habboubi HH, Mahdi N, Abu-Hijleh TM, et al. The relation of vascular endothelial growth factor (VEGF) gene polymorphisms on VEGF levels and the risk of vasoocclusive crisis in sickle cell disease. Eur J Haematol. 2012;89(5):403-409.

60. Redha NA, Mahdi N, Al-Habboubi HH, Almawi WY. Impact of VEGFA -583C > T polymorphism on serum VEGF levels and the susceptibility to acute chest syndrome in pediatric patients with sickle cell disease. Pediatr Blood Cancer. 2014;61(12):2310-2312.

61. Lopes FC, Traina F, Almeida CB, et al. Key endothelial cell angiogenic mechanisms are stimulated by the circulating milieu in sickle cell disease and attenuated by hydroxyurea. Haematologica. 2015;100(6):730-739.

Supplementary Table 1

Baseline Samples	Total	229
	Healthy Child	15
	SCD Total	214
	˪No Treatment	118
	˪Hydroxyurea	72
	˪Chronic Transfusions	24
Follow Up Samples	Total	122
	˪No Treatment	38
	˪Hydroxyurea	41
	˪Chronic Transfusions	17
	˪No Treatment to Hydroxyurea	26

Supplementary Table 2

Factor	All SCD (N=214)	Healthy Child (N = 15)	SCD No Treatment (N= 118)	SCD Chronic Transfusions (N = 24)	SCD Hydroxyurea (N = 72)
sCD40L	32431.0 (815.5-32431.0)	921.3 (322.1-32431.0)	32431.0 (815.5-32431.0)	32431.0 (20580.0-32431.0)	32431.0 (815.5-32431.0)
EGF	124.6 (3.2-1049.0)	7.7 (3.2-246.0)	124.0 (3.2-1049.0)	172.1 (34.6-599.2)	97.0 (3.2-856.2)
Eotaxin	53.0 (4.8-173.2)	17.9 (9.4-67.5)	50.6 (4.8-173.2)	91.1 (30.8-173.2)	47.8 (4.8-156.1)
E-Selectin	134.6 (25.4-396.4)	64.2 (30.1-88.3)	160.8 (32.1-396.4)	126.0 (55.7-186.8)	105.7 (25.4-243.4)
FGF-2	38.0 (8.5-408.6)	38.2 (8.5-66.8)	38.2 (8.5-408.6)	41.2 (19.9-172.2)	30.7 (8.5-147.3)
FKN	32.4 (4.0-4016.0)	66.3 (4.0-407.0)	29.2 (4.0-4016.0)	79.4 (4.0-4016.0)	31.9 (4.0-2947.0)
Flt-3L	4.0 (4.0-590.8)	4.0 (4.0-31.2)	4.0 (4.0-590.8)	4.0 (4.0-590.8)	4.0 (4.0-432.8)
G-CSF	13.8 (3.9-572.8)	21.8 (3.9-69.2)	13.8 (3.9-572.8)	14.4 (3.9-512.3)	13.5 (3.9-233.4)
GM-CSF	8.0 (2.5-219.4)	5.6 (2.5-24.7)	8.4 (2.5-219.4)	7.7 (3.6-97.4)	6.7 (2.5-96.6)
GRO	1634.0 (295.7-5389.0)	96.0 (13.6-2133.0)	1842.5 (295.7-5389.0)	1171.5 (498.1-2146.0)	1486.0 (295.7-5389.0)
sICAM-1	147.2 (37.2-496.3)	98.7 (66.5-236.7)	152.7 (83.0-496.3)	125.4 (54.2-209.9)	145.9 (37.2-496.3)
IFNα2	10.4 (5.4-1620.0)	14.0 (5.4-541.4)	11.3 (5.4-1620.0)	11.9 (5.4-346.2)	9.4 (5.4-283.9)
IFNγ	4.5 (2.0-2793.0)	6.2 (2.0-56.7)	4.5 (2.0-2793.0)	5.0 (2.0-453.2)	4.0 (2.0-2793.0)
IL-1α	4.0 (4.0-393.1)	4.0 (4.0-57.4)	4.0 (4.0-393.1)	4.7 (4.0-57.2)	4.0 (4.0-278.0)
IL-1β	2.8 (2.8-96.9)	2.8 (2.8-21.8)	2.8 (2.8-96.9)	2.8 (2.8-20.0)	2.8 (2.8-34.9)
IL-1RA	16.7 (3.4-1989.0)	35.7 (3.4-78.5)	18.1 (3.4-1989.0)	21.3 (3.4-526.2)	11.8 (3.4-385.0)
IL-2	3.0 (3.0-111.8)	3.0 (3.0-17.6)	3.0 (3.0-111.8)	3.0 (3.0-29.0)	3.0 (3.0-44.6)
IL-3	2.5 (2.5-21.5)	2.5 (2.5-6.1)	2.5 (2.5-21.5)	2.5 (2.5-2.5)	2.5 (2.5-21.5)
IL-4	2.8 (2.8-226.4)	2.8 (2.8-91.0)	2.8 (2.8-226.4)	3.5 (2.8-170.6)	2.8 (2.8-226.4)
IL-5	3.1 (3.1-85.8)	3.1 (3.1-10.3)	3.1 (3.1-85.8)	3.1 (3.1-17.3)	3.1 (3.1-61.1)
IL-6	10.3 (10.3-142.6)	10.3 (10.3-30.4)	10.3 (10.3-142.6)	10.3 (10.3-21.3)	10.3 (10.3-116.7)
IL-7	3.1 (3.0-205.3)	3.0 (3.0-6.3)	3.0 (3.0-205.3)	4.9 (3.0-73.2)	3.0 (3.0-205.3)
IL-8	11.5 (3.1-487.4)	16.2 (3.1-246.0)	10.8 (3.1-487.4)	14.5 (3.1-138.6)	11.4 (3.1-487.4)
IL-9	3.1 (3.1-69.9)	3.1 (3.1-6.2)	3.1 (3.1-69.9)	3.1 (3.1-5.4)	3.1 (3.1-69.9)
IL-10	1.1 (1.0-98.4)	1.0 (1.0-32.4)	1.3 (1.0-98.4)	1.4 (1.0-17.2)	1.0 (1.0-98.4)
IL-12 p40	3.4 (3.4-728.4)	3.4 (3.4-202.3)	3.4 (3.4-728.4)	3.4 (3.4-178.5)	3.4 (3.4-250.0)
IL-12 p70	1.6 (1.6-393.8)	3.1 (1.6-51.4)	1.6 (1.6-393.8)	1.6 (1.6-264.7)	1.6 (1.6-393.8)
IL-13	3.6 (3.6-286.9)	3.6 (3.6-70.6)	3.6 (3.6-286.9)	3.6 (3.6-286.9)	3.6 (3.6-141.1)
IL-15	1.7 (1.7-53.1)	1.7 (1.7-20.1)	1.7 (1.7-53.1)	1.7 (1.7-21.0)	1.7 (1.7-43.3)
IL-17A	3.0 (3.0-1006.0)	3.0 (3.0-31.2)	3.0 (3.0-1006.0)	3.0 (3.0-202.6)	3.0 (3.0-1006.0)
IP-10	218.7 (68.3-1167.0)	82.3 (35.0-385.0)	221.7 (75.9-1167.0)	250.6 (139.1-651.3)	197.3 (68.3-876.8)
MCP-1	177.7 (53.4-1366.0)	130.7 (3.0-354.5)	145.2 (60.8-1366.0)	281.7 (103.0-586.4)	193.6 (53.4-1253.0)
MCP-3	7.6 (4.3-244.1)	15.0 (4.3-160.7)	8.0 (4.3-244.1)	13.4 (4.3-190.0)	5.8 (4.3-143.7)
MDC	1625.0 (12.2-27284.0)	490.8 (266.2-1358.0)	1717.5 (12.2-27284.0)	1728.5 (929.3-5294.0)	1456.0 (12.2-27284.0)
MIP-1α	11.3 (3.2-7339.0)	4.2 (3.2-163.0)	11.0 (3.2-7339.0)	14.8 (3.2-5076.0)	10.0 (3.2-4225.0)
MIP-1β	43.9 (5.0-295.5)	21.1 (5.0-136.0)	45.1 (7.8-295.5)	71.4 (23.2-220.4)	35.0 (5.0-141.4)
TGFα	2.5 (2.5-106.4)	2.5 (2.5-10.8)	3.0 (2.5-106.4)	3.4 (2.5-33.3)	2.5 (2.5-17.7)
TNFα	8.1 (3.0-62.3)	8.6 (3.0-63.8)	9.1 (3.0-62.3)	8.3 (3.0-14.5)	5.9 (3.0-38.1)
TNFβ	3.1 (3.1-451.7)	3.1 (3.1-102.6)	3.1 (3.1-451.7)	3.1 (3.1-301.4)	3.1 (3.1-209.0)
sVCAM-1	862.1 (275.1-3211.9)	535.8 (378.8-943.0)	971.0 (475.5-3211.9)	798.8 (393.8-1346.0)	785.8 (275.1-3211.9)
VEGF	204.3 (16.0-2246.0)	77.0 (16.0-419.4)	219.7 (16.0-2246.0)	268.7 (16.0-1457.0)	122.1 (16.0-2001.0)

Table legend: Summary of medians and ranges for each risk group at baseline sampling. Note, medians were calculated using the limit of detection for each factor for undetectable values. Ranges are given in parentheses.

Supplemental Table 3

	Raw Mann Whitney U	Raw Fisher Exact	FDR-adjusted P value
sCD40L	5.17E-13	8.53572E-09	<.0001
EGF	5.14E-09	0.0004	0.0021
Eotaxin	1.3863E-08	ND	<.0001
sE-selectin	4.7E-10	ND	<.0001
FGF-2	0.3328	ND	0.5685
FKN	0.1336	ND	0.2883
Flt-3L	0.7259	1	1
G-CSF	0.1686	ND	0.3456
GM-CSF	0.0708	ND	0.1814
GRO	1.9E-11	ND	<.0001
sICAM-1	0.0013	ND	0.0053
IFNα2	0.4648	0.3962	0.6271
IFNγ	0.5414	1	1
IL-1α	0.1031	0.104	0.2369
IL-1β	0.5399	0.72	0.9517
IL-1RA	0.2798	ND	0.5214
IL-2	0.6885	1	1
IL-3	0.5845	0.3078	0.5487
IL-4	0.7472	1	1
IL-5	0.5953	0.7014	0.9517
IL-6	0.8957	1	1
IL-7	0.0193	0.0307	0.0839
IL-8	0.9102	ND	1
IL-9	0.8728	0.6714	0.9492
IL-10	0.9486	0.7922	0.9553
IL-12p40	0.4541	0.3984	0.6271
IL-12p70	0.0311	0.0062	0.0212
IL-13	0.5022	0.7788	0.9553
IL-15	0.7054	0.7428	0.9517
IL-17A	0.6408	0.413	0.6271
IP-10	1.4625E-07	ND	<.0001
MCP-1	0.0121	ND	0.0354
MCP-3	0.2081	0.1874	0.3659
MDC	3.3612E-10	ND	<.0001
MIP-1α	0.0885	0.0933	0.225
MIP-1β	0.004882	ND	0.0182
TGFα	0.0027	0.0007	0.0032
TNFα	0.8944	ND	1
TNFβ	0.7689	0.5681	0.8319
sVCAM-1	1.158E-08	ND	<.0001
VEGF	0.001	0.0082	0.0259

Table Legend: Raw and false discover rate (FDR) adjusted p-values for both Mann Whitney U and Fisher Exact tests on paired data. A fisher exact test was performed on all samples where >25% were outside the limit of detection (LOD). The test was performed based on the number of samples above versus below the LOD. Significant differences ($p<0.05$) are highlighted in grey. False discovery rate (FDR) correct p-values are given for Mann Whitney U test or Fisher Exact test if run. ND: not done.

Supplementary Table 4

Cytokines	Brief Descriptions	References
sCD40L	Circulating soluble CD40 ligand (sCD40L) can be produced by activated platelets and T cells. It induces B cell proliferation. High levels of sCD40L are often associated with inflammatory disorders, and can be responsible for adverse events associated with transfusions.	(9-13)
EGF	Epidermal growth factor (EGF) can be produced by endothelial cells. It is a mitogen that stimulates the proliferation of fibroblasts and epithelial cells. It is associated with wound healing and can stimulate hematopoietic regeneration after injury.	(14, 15)
Eotaxin	Eotaxin (CCL-11) is produced by endothelial cells, monocytes, epithelial cells, and T cells. This chemokine attracts eosinophils, monocytes, and T cells.	(16, 17)
E-Selectin	E-selectin (CD62E) is expressed by activated endothelial cells. It is a cell adhesion molecule and sugar-binding lectin. It can bind granulocyes including neutrophils and eosinophils, and often associates with tissue inflammation.	(18-21)
FGF-2	Fibroblast growth factor-2 (FGF-2) can serve as an angiogenic and hematopoietic growth factor. It can be expressed by bone marrow stromal cells and trigger stromal cells and hematopoietic progenitors.	(22, 23)
FKN	Fracktalkine (FKN or CX3CL1) is expressed by endothelial cells, macrophages, dendritic cells, and fibroblasts. Soluble chemokine attracts T cells, NK cells, and monocytes. This chemokine associates with neuron-microglial interactions, leukocyte adhesion, and chronic inflammation.	(24-26)
GRO	Growth-regulated oncogene (GRO) is a chemotactic cytokine, which can be produced by activated monocytes, endothelial cells and fibroblasts. It has neutrophil and basophil-stimulating activities.	(27)
sICAM-1	Soluble intercellular adhesion molecule 1 (sICAM-1) is produced by endothelial cells and leukocytes, often in response to infection or cell damage. It is up-regulated in several disease states and is able to prevent the cellular infection and replication of some viruses.	(11, 20, 28-32)
IFNα2	Interferon α2 (IFNα2) is produced by many different cell types in response to infections and cell damage. Receptors are ubiquitously expressed, so that potential cell targets are diverse, including lymphocytes, dendritic cells and NK cells. Anti-tumor and anti-viral states are generated by this factor.	(33, 34)
IL-4	Interleukin 4 (IL-4) is produced by T cells, mast cells and basophils. It supports the production of Th2 cells, which in turn support antibody production and modulate isotype expression by B cells.	(35)
IL-7	Interleukin 7 (IL-7) is produced by bone marrow stromal cells. It is also produced by dendritic cells, neurons and epithelial cells. It supports the early differentiation and development of pre-B cells and pre-T cells, as well as the development of secondary lymphoid tissues. IL-7 also affects CD8 T cell development, consequent CD4/CD8 ratios, and interactions between T cells and dendritic cells. IL-7 regulates cell apoptosis, activation, proliferation, and survival.	(36)
IL-12(p70)	Interleukin 12 (IL-12) is produced by B cells, macrophages, and dendritic cells. It supports Th1 cell development, NK cell activation, and the cytotoxic T cell response.	(37)
IP-10	Interferon γ-inducible protein (IP-10 or CXCL10) is produced by monocytes, macrophages, fibroblasts, endothelial cells, and epithelial cells including keratinocytes, often in response to cytokine stimulation. This chemokine attracts T cells, NK cells, and monocytes, and promotes Th1 activity. IP-10 can inhibit the proliferation of endothelial cells, vascularization, wound healing, and bacterial growth.	(38, 39)
MCP-1	Monocyte chemotactic protein (MCP-1 or CCL2) is produced by monocytes, macrophages, fibroblasts and keratinocytes. This chemokine activates macrophages, induces histamine release by basophils, and promotes Th2 activities.	(15, 40)
MDC	Macrophage-derived chemokine (MDC or CCL22) is produced by macrophages and dendritic cells. This chemokine	(41, 42)

	recruits targets including dendritic cells, NK cells, and T cells, particularly of the Th2 subset.	
MIP-1α	Macrophage inflammatory protein 1α (MIP-1α or CCL3) is produced by macrophages, monocytes, T cells, mast cells, and fibroblasts. This chemokine activates and recruits lymphocytes, NK cells, dendritic cells, monocytes, and granulocytes including neutrophils and basophils. It up-regulates IFN-γ secretion by activated T cells, which further induces Th1 cell functions. MIP-1 α can also inhibit or alter the proliferation of hematopoietic stem cells.	(43, 44)
MIP-1β	Macrophage inflammatory protein 1β (MIP-1β or CCL4) is produced by macrophages, monocytes, T cells, neutrophils and endothelial cells. This chemokine attracts monocytes, lymphocytes, NK cells, and dendritic cells to sites of injury.	(45, 46)
TGFα	Transforming growth factor α (TGFα) is a mitogenic protein that can be produced by epithelial cells, and can serve as a ligand for the epidermal growth factor receptor. The protein induces cell proliferation and differentiation.	(47, 48)
TNFα	Tumor necrosis factor α (TNFα) is produced by macrophages, monocytes, neutrophils, T cells, and NK cells. TNF regulates the growth/necrosis and function of fibroblasts, neutrophils, and a variety of tumor cells.	(49-53)
sVCAM-1	Soluble vascular cell adhesion molecule-1 (sVCAM-1) is produced by endothelial cells. It mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils. Sickled erythrocytes also adhere to this factor. The factor associates with oxidative stress and vascular inflammation.	(50, 54-57)
VEGF	Vascular endothelial growth factor (VEGF) is produced by endothelial cells and pericytes, often in response to hypoxia. This factor regulates angiogenesis and the permeability and neuropathy of blood vessels.	(15, 58-61)

Supplementary Table 5

	Kruskal-Wallis Anova		Welch's Anova	
	Raw	FDR Adjusted	Raw	FDR Adjusted
sCD40L	2.36544E-13	<.0001	3.27187E-10	<.0001
EGF	4.33094E-07	<.0001	7.03293E-08	<.0001
Eotaxin	8.26772E-10	<.0001	7.50302E-09	<.0001
sE-selectin	1.9523E-13	<.0001	4.30076E-13	<.0001
FGF-2	0.023989231	0.0466	0.019950381	0.0389
FKN	0.018659871	0.0385	0.02001881	0.0389
Flt-3L	ND	ND	ND	ND
G-CSF	0.532736676	0.7032	0.497506388	0.7138
GM-CSF	0.219354125	0.3619	0.117934241	0.2162
GRO	8.94299E-11	<.0001	1.09654E-09	<.0001
sICAM-1	0.000164951	0.0004	0.000718241	0.0017
IFNα2	0.666895276	0.7851	0.842223158	0.9325
IFNγ	0.689907385	0.7851	0.853574025	0.9325
IL-1α	0.256539611	0.3896	0.288108346	0.5004
IL-1β	ND	ND	ND	ND
IL-1RA	0.580121598	0.7363	0.726136027	0.8875
IL-2	ND	ND	ND	ND
IL-3	ND	ND	ND	ND
IL-4	0.626535664	0.7658	0.600575269	0.8258
IL-5	ND	ND	ND	ND
IL-6	ND	ND	ND	ND
IL-7	0.001808123	0.004	9.55454E-05	0.0003
IL-8	0.39193364	0.5389	0.465249753	0.6979
IL-9	ND	ND	ND	ND
IL-10	0.175569934	0.3049	0.893475495	0.9325
IL-12p40	0.7852875	0.793	0.932513501	0.9325
IL-12p70	0.145055414	0.2659	0.829183687	0.9325
IL-13	0.793025732	0.793	0.710879042	0.8875
IL-15	ND	ND	ND	ND
IL-17A	0.774178054	0.793	0.721395524	0.8875
IP-10	1.38885E-05	<.0001	2.94785E-05	<.0001
MCP-1	2.78544E-08	<.0001	6.976E-07	<.0001
MCP-3	0.349735828	0.5018	0.440825225	0.6927
MDC	5.84173E-08	<.0001	1.01205E-09	<.0001
MIP-1α	0.259741594	0.3896	0.346138477	0.5711
MIP-1β	1.99388E-06	<.0001	1.15641E-05	<.0001
TGFα	1.11611E-07	<.0001	0.000854256	0.0019
TNFα	9.77009E-06	<.0001	0.000295684	0.0008
TNFβ	0.747972176	0.793	0.907853805	0.9325
sVCAM-1	1.95928E-10	<.0001	2.64985E-10	<.0001
VEGF	5.81443E-05	0.0001	0.00017525	0.0005

Table Legend: Raw and false discovery rate (FDR) adjusted p-values for both Kruskal-Wallis and Welch's Anovas performed on baseline data. Significant differences ($p<0.05$) are highlighted in grey. Anova analysis was not performed on factors where >75% of all values were below the limit of detection. ND: not done**.**

Supplementary Table 6a

	NT → HU (N = 26)		NT → NT (N = 38)	
	Baseline	Follow Up	Baseline	Follow Up
sCD40L	**32431.0 (18159.0-32431.0)**	**30587.0 (734.9-32431.0)****	32431.0 (10325.0-32431.0)	32431.0 (5206.0-32431.0)
EGF	**120.3 (10.3-294.2)**	**58.0 (8.4-321.6)***	143.4 (15.1-626.2)	115.5 (5.9-791.7)
Eotaxin	49.2 (27.8-104.3)	51.5 (24.2-110.8)	50.7 (21.0-154.9)	54.7 (21.0-142.7)
E-Selectin	**156.6 (62.7-396.4)**	**108.6 (33.5-190.0)*****	153.9 (60.4-344.8)	152.5 (53.4-351.3)
FGF-2	41.4 (9.3-128.1)	39.8 (8.5-84.8)	39.5 (8.5-184.2)	40.0 (8.5-205.5)
FKN	44.8 (4.0-3983.0)	53.7 (4.0-892.2)	21.9 (4.0-468.6)	35.2 (4.0-1356.0)
Flt-3L	4.0 (4.0-354.3)	4.0 (4.0-177.0)	4.0 (4.0-55.4)	4.0 (4.0-98.3)
G-CSF	16.8 (3.9-106.7)	20.7 (3.9-113.1)	13.0 (3.9-145.3)	14.2 (3.9-166.7)
GM-CSF	10.0 (3.3-42.5)	9.9 (2.5-51.2)	8.3 (2.5-77.4)	8.2 (2.5-162.4)
GRO	1654.0 (516.5-3774.0)	1434.0 (167.4-2224.0)	1795.5 (397.2-3117.0)	1674.0 (350.3-3939.0)
sICAM-1	143.2 (83.0-200.8)	147.2 (75.4-345.6)	158.9 (92.2-247.3)	151.6 (80.1-296.7)
IFNα2	20.6 (5.4-1620.0)	14.8 (5.4-608.5)	11.7 (5.4-342.2)	13.9 (5.4-443.4)
IFNγ	7.1 (2.0-418.7)	11.6 (2.0-112.7)	4.4 (2.0-136.6)	6.0 (2.0-847.7)
IL-1α	4.3 (4.0-167.7)	4.0 (4.0-268.5)	4.0 (4.0-81.2)	4.0 (4.0-81.2)
IL-1β	2.8 (2.8-65.1)	2.8 (2.8-53.1)	2.8 (2.8-14.6)	2.8 (2.8-16.0)
IL-1RA	31.6 (3.4-167.6)	18.1 (3.4-198.7)	21.4 (3.4-376.8)	19.2 (3.4-1023.0)
IL-2	3.0 (3.0-17.8)	3.0 (3.0-19.0)	3.0 (3.0-16.6)	3.0 (3.0-18.0)
IL-3	2.5 (2.5-9.6)	2.5 (2.5-9.9)	2.5 (2.5-3.7)	2.5 (2.5-6.6)
IL-4	2.8 (2.8-224.0)	2.8 (2.8-216.6)	2.8 (2.8-169.7)	2.8 (2.8-166.6)
IL-5	3.1 (3.1-16.1)	3.1 (3.1-13.6)	3.1 (3.1-20.5)	3.1 (3.1-20.2)
IL-6	10.3 (10.3-49.8)	10.3 (10.3-31.7)	10.3 (10.3-142.6)	10.3 (10.3-27.0)
IL-7	**3.7 (3.0-104.3)**	**3.0 (3.0-78.8)***	3.0 (3.0-69.3)	3.0 (3.0-40.0)
IL-8	13.8 (4.5-116.8)	17.9 (3.1-69.1)	10.8 (3.1-93.1)	12.8 (3.1-358.7)
IL-9	3.1 (3.1-5.5)	3.1 (3.1-4.4)	3.1 (3.1-8.8)	3.1 (3.1-13.8)
IL-10	1.4 (1.0-14.1)	1.0 (1.0-20.4)	1.4 (1.0-20.3)	1.0 (1.0-26.3)
IL-12 p40	3.4 (3.4-161.6)	3.4 (3.4-127.9)	3.4 (3.4-213.9)	3.4 (3.4-289.5)
IL-12 p70	1.7 (1.6-235.9)	2.0 (1.6-10.6)	1.6 (1.6-123.3)	1.6 (1.6-47.5)
IL-13	3.6 (3.6-191.4)	3.6 (3.6-132.9)	3.6 (3.6-111.3)	3.6 (3.6-93.5)
IL-15	1.7 (1.7-20.8)	1.7 (1.7-25.7)	1.7 (1.7-13.7)	1.7 (1.7-15.8)
IL-17A	3.8 (3.0-128.7)	5.0 (3.0-56.1)	3.0 (3.0-32.3)	3.0 (3.0-109.0)
IP-10	232.9 (144.4-1167.0)	235.1 (137.5-962.6)	222.5 (87.4-868.4)	206.2 (66.6-819.3)
MCP-1	**130.7 (66.0-431.6)**	**209.4 (86.8-673.1)*****	152.4 (67.4-1366.0)	155.6 (68.4-570.7)
MCP-3	23.6 (4.3-163.1)	14.4 (4.3-157.9)	9.2 (4.3-178.7)	8.3 (4.3-396.0)
MDC	1572.0 (12.2-5274.0)	1519.0 (15.5-3383.0)	2064.5 (530.4-5710.0)	1935.0 (458.7-13909.0)
MIP-1α	**12.1 (3.2-4490.0)**	**7.9 (3.2-3920.0)***	11.6 (3.2-7339.0)	12.7 (3.2-7339.0)
MIP-1β	**44.1 (18.1-295.5)**	**34.6 (5.0-91.7)*****	46.4 (13.9-112.5)	49.2 (6.9-423.9)
TGFα	3.3 (2.5-13.0)	2.5 (2.5-11.2)	2.8 (2.5-104.4)	2.5 (2.5-175.0)
TNFα	9.5 (4.3-40.4)	8.6 (3.0-37.1)	10.2 (3.3-30.2)	9.8 (3.0-36.5)
TNFβ	3.1 (3.1-209.0)	3.1 (3.1-220.2)	3.1 (3.1-237.2)	3.1 (3.1-537.1)
sVCAM-1	**890.0 (478.5-2238.9)**	**801.0 (289.3-1208.1)****	942.8 (545.3-1583.9)	901.1 (474.4-1383.4)
VEGF	228.6 (16.0-1606.0)	191.0 (16.0-783.3)	164.9 (18.6-1383.0)	191.7 (16.0-1925.0)

Table Legend: Medians and ranges for each risk group at baseline and follow up sampling. Medians are shown with ranges in parenthesis. Statistically significant differences are bolded and marked with an asterisk(s). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Table 6b

	HU → HU (N = 41)		CT → CT (N = 17)	
	Baseline	Follow Up	Baseline	Follow Up
sCD40L	32431.0 (8951.0-32431.0)	32431.0 (13251.0-32431.0)	32431.0 (20580.0-32431.0)	32431.0 (3703.0-32431.0)
EGF	124.3 (9.4-856.2)	127.3 (14.8-638.5)	177.0 (34.6-599.2)	160.7 (29.9-1224.0)
Eotaxin	**48.0 (18.1-156.1)**	**61.4 (20.6-215.6)****	100.9 (31.4-173.2)	87.8 (48.2-225.6)
E-Selectin	107.5 (54.6-243.4)	93.4 (27.9-290.0)	118.2 (55.7-186.8)	108.4 (57.7-218.0)
FGF-2	30.7 (8.5-127.5)	31.6 (8.5-114.8)	47.4 (26.7-172.2)	54.3 (18.9-224.9)
FKN	31.4 (4.0-2947.0)	18.6 (4.0-3555.0)	84.4 (4.0-4016.0)	74.4 (10.7-1348.0)
Flt-3L	4.0 (4.0-432.8)	4.0 (4.0-442.7)	4.0 (4.0-302.2)	4.0 (4.0-341.2)
G-CSF	11.0 (3.9-233.4)	12.2 (3.9-429.0)	14.4 (3.9-512.3)	20.0 (3.9-558.5)
GM-CSF	7.1 (2.5-33.2)	4.7 (2.5-39.7)	7.9 (3.9-97.4)	7.5 (2.5-143.3)
GRO	**1677.0 (816.8-5389.0)**	**1826.0 (934.1-2916.0)***	1096.0 (498.1-2146.0)	1248.0 (520.6-2168.0)
sICAM-1	143.1 (85.3-463.3)	146.0 (70.3-253.9)	126.1 (54.2-175.2)	139.5 (79.1-251.7)
IFNα2	**8.5 (5.4-149.9)**	**5.4 (5.4-115.4)*****	13.9 (5.4-346.2)	12.2 (5.4-393.0)
IFNγ	3.8 (2.0-2793.0)	4.7 (2.0-162.9)	5.0 (2.0-175.8)	5.9 (2.0-166.8)
IL-1α	4.0 (4.0-278.0)	4.0 (4.0-228.1)	4.0 (4.0-57.2)	4.0 (4.0-79.9)
IL-1β	2.8 (2.8-17.2)	2.8 (2.8-11.0)	2.8 (2.8-10.6)	2.8 (2.8-12.7)
IL-1RA	14.8 (3.4-318.8)	12.3 (3.4-196.2)	21.3 (3.4-526.2)	27.6 (3.4-807.9)
IL-2	3.0 (3.0-17.2)	3.0 (3.0-9.9)	3.0 (3.0-29.0)	3.0 (3.0-37.9)
IL-3	2.5 (2.5-8.8)	2.5 (2.5-2.5)	2.5 (2.5-2.5)	2.5 (2.5-5.6)
IL-4	**2.8 (2.8-149.8)**	**2.8 (2.8-75.3)*****	4.2 (2.8-170.6)	2.8 (2.8-189.8)
IL-5	3.1 (3.1-7.7)	3.1 (3.1-6.4)	3.1 (3.1-17.3)	3.1 (3.1-23.6)
IL-6	10.3 (10.3-86.5)	10.3 (10.3-10.3)	10.3 (10.3-21.3)	10.3 (10.3-21.0)
IL-7	**3.0 (3.0-205.3)**	**3.0 (3.0-62.1)****	5.3 (3.0-73.2)	3.0 (3.0-51.3)
IL-8	11.2 (3.1-487.4)	11.8 (3.1-63.4)	14.8 (3.3-76.9)	20.7 (3.1-67.1)
IL-9	3.1 (3.1-69.9)	3.1 (3.1-27.3)	3.1 (3.1-5.4)	3.1 (3.1-7.5)
IL-10	1.0 (1.0-28.5)	1.0 (1.0-96.0)	1.7 (1.0-17.2)	2.4 (1.0-16.3)
IL-12 p40	3.4 (3.4-135.5)	3.4 (3.4-74.6)	3.4 (3.4-178.5)	3.4 (3.4-278.2)
IL-12 p70	1.6 (1.6-393.8)	1.6 (1.6-120.6)	1.6 (1.6-264.7)	1.9 (1.6-42.6)
IL-13	3.6 (3.6-108.6)	3.6 (3.6-68.8)	3.6 (3.6-136.3)	4.8 (3.6-158.9)
IL-15	1.7 (1.7-19.1)	1.7 (1.7-4.3)	1.7 (1.7-21.0)	1.7 (1.7-20.2)
IL-17A	3.0 (3.0-1006.0)	3.0 (3.0-83.6)	3.0 (3.0-53.3)	3.0 (3.0-35.8)
IP-10	213.3 (68.3-876.8)	223.1 (80.1-2435.0)	251.5 (139.1-612.8)	277.9 (160.8-1081.0)
MCP-1	193.8 (53.4-752.9)	223.4 (77.3-832.3)	277.0 (155.4-586.4)	276.4 (99.2-1028.0)
MCP-3	5.8 (4.3-110.5)	4.3 (4.3-99.5)	13.4 (4.3-190.0)	12.1 (4.3-214.0)
MDC	**1445.0 (275.4-27284.0)**	**1875.0 (292.1-4705.0)*****	1706.0 (929.3-3111.0)	1763.0 (260.1-2673.0)
MIP-1α	10.2 (3.2-4083.0)	5.5 (3.2-2588.0)	14.1 (3.2-5076.0)	12.9 (3.2-4035.0)
MIP-1β	34.6 (5.0-141.4)	33.0 (6.2-130.2)	58.4 (23.2-127.7)	61.8 (5.0-165.8)
TGFα	2.5 (2.5-17.7)	2.5 (2.5-12.5)	3.4 (2.5-33.3)	3.7 (2.5-48.3)
TNFα	**5.9 (3.0-38.1)**	**9.0 (3.0-24.5)*****	**8.4 (3.0-14.5)**	**9.6 (4.1-22.2)***
TNFβ	3.1 (3.1-125.7)	3.1 (3.1-129.9)	3.1 (3.1-301.4)	5.0 (3.1-360.2)
sVCAM-1	**800.3 (275.1-3211.9)**	**701.6 (290.5-1217.1)*****	794.2 (399.2-1146.5)	740.2 (490.5-1676.2)
VEGF	118.0 (16.0-2001.0)	124.8 (16.0-1100.0)	253.5 (16.0-1060.0)	214.4 (29.1-1277.0)

Table Legend: Medians and ranges for each risk group at baseline and follow up sampling. Medians are shown with ranges in parenthesis. Statistically significant differences are bolded and marked with an asterisk(s). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Table 7

Time Point	Treatment Group	C-Reactive Protein (mg/dL)			D-Dimer (mcg/mL)			Factor VIII Antigen level (%)			Platelet Count (x10^9/dL)			vWF:Ag (%)			WBC (x10^9/dL)		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Baseline	*Continuous hydroxyurea*	43	0.3	0.3	43.0	1.2	1.2	51.0	255.5	66.7	41.0	347.8	148.0	43.0	176.8	49.7	41.0	7.8	2.9
	No treatment	38	0.4	0.5	38.0	1.2	0.9	44.0	250.0	65.9	38.0	395.9	106.7	38.0	157.1	50.0	38.0	11.9	3.1
	Initiated hydroxyurea	26	0.7	1.4	26.0	1.6	0.9	35.0	284.8	60.2	26.0	357.0	97.5	25.0	186.5	58.1	26.0	11.5	3.7
	Continuous chronic transfusions	16	0.2	0.2	17.0	0.6	0.3	18.0	206.3	76.8	19.0	360.0	109.2	17.0	145.7	55.2	19.0	13.1	3.5
	Total	123	0.4	0.7	124.0	1.2	1.0	148.0	254.8	69.3	124.0	366.3	121.0	123.0	168.4	53.6	124.0	10.7	3.8
Year 2	*Continuous Hydroxyurea*	43	0.6	1.4	43.0	1.3	0.9	50.0	259.9	70.2	41.0	375.1	156.0	42.0	177.0	58.3	41.0	8.3	3.0
	No Treatment	37	0.4	0.4	36.0	1.3	0.8	39.0	255.7	61.2	37.0	391.3	117.2	36.0	161.6	48.0	37.0	12.3	4.5
	Initiated hydroxyurea	26	0.4	0.4	26.0	1.6	0.9	33.0	270.1	68.6	26.0	**291.92*****	100.7	26.0	**167.73****	61.6	26.0	**7.93*****	2.7
	Continuous chronic transfusions	16	0.2	0.2	17.0	0.6	0.4	17.0	205.5	72.9	22.0	339.1	101.1	17.0	160.9	64.2	22.0	14.3	4.9
	Total	122	0.5	0.9	122.0	1.3	0.9	139.0	254.5	69.7	126.0	356.4	129.9	121.0	168.2	56.8	126.0	10.5	4.6

Table Legend: vWF:Ag = Von Willebrand antigen level, WBC = white blood cell count. **p<0.01, ***p<0.001.

Supplementary Table 8

	No. of patients	Incidence rate* of VOC events in the 2 years prior to baseline (per 100 person-years)	Incidence rate* of VOC events in the 2 years after baseline (per 100 person-years)	P-value
Continuous hydroxyurea	41	15.9	4.9	**0.01**
Initiated hydroxyurea	26	21.2	5.8	**0.009**
No treatment	38	10.5	6.6	0.37
Continuous transfusions	17	8.8	2.9	0.33

*Incidence rate = Number of events/number of patients/2*100. VOC: vaso-occlusive events