


Volume 101 | Number 11 | November 2016


Jump To

- Article
- Acknowledgments
- Footnotes
- References
- Figures & Data
- Info & Metrics
- PDF

EHA



EUROPEAN
HEMATOLOGY
ASSOCIATION



Journal of the European Hematology Association
Owned & published by the Ferrata Storti Foundation

Advanced Search

Home Current Issue Ahead Of Print Archive Submit a Manuscript

About Us More

Mutating Heme Oxygenase-1 Into A Peroxidase Causes A Defect In Bilirubin Synthesis Associated With Microcytic Anemia And Severe Hyperinflammation

Johann Greil, Maria V. Verga-Falzacappa, Nicole E. Echner, Wolfgang Behnisch, Obul R. Bandapalli, Paulina Pechanska, Stephan Immenschuh, Vijith Vijayan, Jozsef Balla, Hirokatsu Tsukahara, Marion Schneider, Gritta Janka, Maren Claus, Thomas Longerich, Martina U. Muckenthaler, Andreas E. Kulozik

Haematologica November 2016 101: e436-e439; **Doi:**10.3324/haematol.2016.147090

AUTHOR AFFILIATIONS

Johann Greil¹, Maria V. Verga-Falzacappa¹, Nicole E. Echner¹, Wolfgang Behnisch¹, Obul R. Bandapalli¹, Paulina Pechanska¹, Stephan Immenschuh², Vijith Vijayan², Jozsef Balla^{3,4}, Hirokatsu Tsukahara⁵, Marion Schneider⁶, Gritta Janka⁷, Maren Claus⁸, Thomas Longerich⁹, Martina U. Muckenthaler^{1,*†} and Andreas E. Kulozik^{1,*†}

¹Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Germany

²Institute for Transfusion Medicine, Hannover Medical School, Germany

³Department. of Nephrology, Medical and Health Science Center, University of Debrecen, Hungary

⁴MTA-DE Vascular Biology, Thrombosis and Hemostasis Research Group, Hungarian Academy of Sciences Debrecen, Hungary

⁵Department of Pediatrics, Okayama University Graduate School of Medicine, Japan

⁶Section of Experimental Anesthesiology, University of Ulm, Germany

⁷Department of Hematology and Oncology, Children's Hospital, University of Hamburg, Germany

⁸Institute for Immunology, University Heidelberg, Germany

⁹Institute of Pathology, University of Heidelberg, Germany

Correspondence: andreas.kulozik@med.uni-heidelberg.de/martina.muckenthaler@med.uni-heidelberg.de

Article

Figures & Data

Info & Metrics

PDF

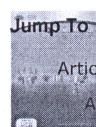
Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder characterized by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hypercytokinemia, and hemophagocytosis. The genetically determined forms of HLH, also referred to as familial hemophagocytic lymphohistiocytosis, commonly manifest in infants or in young children and are characterized by genetic abnormalities of natural killer (NK)-cell function. HLH may also develop secondary to inborn errors of metabolism.^{1,2}

Heme oxygenases are the first and rate-limiting enzymes of heme degradation. Heme oxygenase-1 (HO-1) is ubiquitously expressed at low levels, but is highly inducible in response to stress.³ The cytoprotective, antioxidant and anti-inflammatory roles of HO-1 are mainly attributed to the elimination of free heme, thus limiting the generation of reactive oxygen species (ROS), but also to the generation of the protective products of heme degradation carbon monoxide, biliverdin, and bilirubin.⁴

We describe a patient carrying a G139V mutation of the active center of HO-1 resulting in loss of normal HO-1 function and gain in pathological peroxidase activity. The patient showed increased urinary excretion of *in vivo* markers for lipid peroxidation, oxidative protein and DNA damage, oxidative glycation and oxidative bilirubin metabolites. Functional studies of peripheral blood mononuclear cells revealed exquisite oxidative stress sensitivity and lipopolysaccharide hyper-responsiveness. Clinically, the mutation was associated with hemophagocytic lymphohistiocytosis (HLH), a severe inflammatory disorder hallmarked by excessive macrophage activation and abnormal NK-cell function. This distinguishes the phenotype from two published pediatric cases caused by HO-1 null-mutations with loss of enzyme function, which are not associated with HLH.^{5,6} We show that the disease is clinically modulated by limiting heme challenge through calculated iron depletion, thus highlighting the importance of HO-1 function in oxidative stress defense and in the regulation of the macrophage-dependent inflammatory response.

The patient is the son of consanguineous Turkish parents. At the age of three months, he developed microcytic anemia and progressive splenomegaly. At 8 months, oral iron treatment

Vol 101 Issue 11



Jump To
 Article
 Acknowledgments
 About the Cover
 Footnotes
 References
 Index by author
 Figures & Data
 Info & Metrics
 PDF

Email
 Print
 Citation
 Tools
 © Request Permissions
 Share

Tweet

Tetszik 0

- Alert me when this article is cited
- Alert me if a correction is posted

More in this TOC Section

Related Articles

No similar articles are available.

PubMed Google Scholar

Cited By...



progressive hepatosplenomegaly (see Table 1 for results of clinical laboratory assessment). Liver histology showed severe hemophagocytosis and Kupffer cell siderosis plus extramedullary hematopoiesis and slight hemophagocytosis was observed in the bone marrow. At 20 months, immunochemotherapy (HLH 2004 protocol⁷) was started and resulted in sustained remission of the clinical and laboratory signs of HLH. Remarkably, IL-1 β , IL-6, TNF- α , ferritin and CRP remained highly elevated indicating ongoing inflammation, whilst the increased sCD25 level normalized rapidly (Table 1). DNA sequence analysis excluded known mutations as potential causes of familial HLH.

Jump To

Article

Acknowledgments

Footnotes

References

Figures & Data

Info & Metrics

PDF

[Collapse inline](#) | [View popup](#) | [Download powerpoint](#)

Table 1.

Clinical and laboratory features of the patient with HO-1 G139V.

| Antibiotic treatment | 0 to 4 weeks post-treatment or second recurrent infection | 7 to 12 weeks post-treat | 4 to 12 months post-treatment or last exacerbation | 20 to 24 months post-treatment or last exacerbation with pharyngeal and cryptogenic A.S. and last exacerbation | 40 to 74 months post-treatment or last exacerbation | 72 to 124 months post-treatment or last exacerbation |
|--------------------------------------|---|--------------------------------|---|--|--|---|
| Penicillin (mm) | absent | absent | present | absent | absent | absent |
| Hypoglycaemic therapy (mg/kg/day) | n.d. | n.d. | present | present | present | absent |
| Hb (g/L)* | 104-148 | 82-103 | 60-121 | 63-127 | 57-165 | 119-161 |
| MCV (fl) | 79-91 | 78-93 | 68-122 | 62-125 | 70-165 | 65-135 |
| Platelet count (x10 ⁹) | 27-39 | 20-96 | 20-92 | 100-428 | 108-468 | 20-128 |
| APG (dL) | 65-80 | 42-78 | 10-101 | 12-109 | 49-116 | 52-125 |
| Serum bilirubin (mg/dL) | 82-114 | n.d. | 0-02 | n.d. | 0-15 | 0-7 |
| LDH (U/L) | 1078-1490 | 852-1408 | 1,027-1618 | 808-1571 | 1024-1367 | 1212-1857 |
| Renal function (mg/dL) | n.d. | n.d. | n.d. | 0.7-1.01 | 0.7-2.06 | 0.8-2.16 |
| Proteinuria (mg/dL) | 21-29 | 17-55 | 1-5 | 1-30 | 0-50 | 0-10 |
| Neutrophils (per cent WBC) | 1-95 | n.d. | 0-54 | 0-101 | 0-30 | 0-101 |
| Lymphocytes (fl) | n.d. | n.d. | 0-61 | 0-16 | 0-16 | 0-107 |
| Thrombocytes (fl) | n.d. | n.d. | 200-1131 | 225-390 | 36-205 | 111-161 |
| Platelets (x10 ⁹) | n.d. | 122-114 | 215-59 | 137-341 | 212-59 | 122-161 |
| Haematocrit (%) | n.d. | n.d. | 0-72 | 11-142 | 52-84 | 15-112 |
| Transferrin saturation (%) | n.d. | n.d. | 22-81 | 5-113 | 5 | n.d. |
| Transferrin (g/L) | n.d. | n.d. | 2-612 | 2-108 | 225 | n.d. |
| uFcr (mg) | n.d. | n.d. | n.d. | 0-35 | 119-114 | n.d. |
| uFcr (mg/L) | 22-50 | 2-103 | 2-103 | 2-103 | 21-102 | 2-103 |
| Cr (mg/dL) | 40-61 | 17-111 | 25-120 | 25-120 | 40-105 | 10-127 |
| Dilution (mg/L) | 14 | n.d. | n.d. | 0-1 | 0-17-5 | n.d. |
| IL-1 (pg/mL) | n.d. | n.d. | 8-8 | 42 | 7-83 | 64 |
| IL-6 (pg/mL) | n.d. | n.d. | 0-620 | 10-149 | 83-102 | 12-8 |
| TN- α (pg/mL) | n.d. | n.d. | 105-424 | 612-150 | 603-103 | 100-8 |
| IL-10 (pg/mL) | n.d. | n.d. | 166-106 | 85-182 | 80-130 | 100-8 |

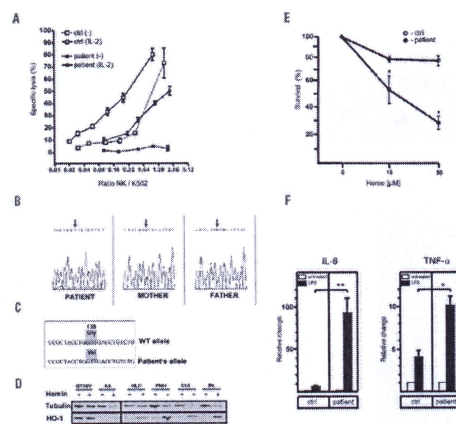
[illegible]

In vitro NK-cell function, measured at age 39 and 45 months, showed decreased killing activity in unstimulated cells, but appropriate killing following IL-2 stimulation (Figure 1A). We tapered immunochemotherapy after 14 months, but four weeks later the patient showed inflammatory activity flare-up (CRP 122 mg/l). CSA was therefore re-started. With improved understanding of the disease pathophysiology,

iron depletion was started by periodical low volume phlebotomy; this resulted in the improvement of anemia and complete resolution of liver fibrosis, Kupffer cell siderosis, and hemophagocytosis. At age 10 years, the patient is currently off immunosuppression with no clinically apparent hyperinflammation. However, elevation of vWF, CRP and D-dimers indicate an ongoing inflammatory vascular process.

Jump To

Article
Acknowledgments
Footnotes
References
Figures & Data
Info & Metrics
PDF



[Download figure](#) | [Open in new tab](#) |

[Download powerpoint](#)

Figure 1.

Genetic and functional characterisation of HO-1 G139V. (A) Impaired cytotoxic activity of the patient's NK cells. Whole blood samples of healthy individuals and the patient were treated with or without 100 U / ml IL-2 overnight at 37 °C. NK-cell activity was then determined by a 4 h ^{51}Cr release assay against the NK-cell sensitive target cell K562. All experiments were performed in triplicate. Data are presented as mean \pm SD. Ctrl, control; NK, natural killer cell. (B) DNA sequence of nucleotides 405 to 427 of the HO-1 coding sequence including the GGT to GTT mutation of codon 139, which was found homozygously in the patient and heterozygously in his parents. (C) The mutation identified in the patient causes the replacement of a glycine by a valine at position 139 in the HO-1 catalytic center. (D) HO-1 and tubulin immunoblot of protein isolated from PBMC of the G139V mutated patient, a normal control (Ctrl) and control patients with aplastic anemia (AA), genetic HLH with underlying immunodeficiency (HLH), paroxysmal nocturnal hemoglobinuria (PNH), and pyruvate kinase deficiency (PK). The PBMC were cultured for 4 hours post-isolation, in the absence (lanes 1, 3, 5, 7, 9, 11) and in the presence (lanes 2, 4, 6, 8, 10, 12) of 100 μM hemin. (E) PBMC of the patient (closed circles) and healthy controls (open circles) were isolated and cultured with the indicated concentrations of heme (10 μM and 50 μM). Dead or apoptotic cells were identified by annexin V staining. Percent survival was determined and compared to cells cultured in medium without heme. Each point represents the mean \pm SD of 3 independent experiments. Statistics, Student's *t*-test for unpaired values: significant differences patient vs. control, * = $P < 0.05$. (F) PBMC of the patient and healthy controls were isolated and cultured as described in the Methods section. After overnight culture, medium was replaced with medium containing either LPS (1 $\mu\text{g}/\text{ml}$; solid bars) or no LPS (open bars) for another 24 h. Concentrations of IL-6 and TNF- α were measured in the supernatant using an ELISA and were normalized to cell number. Values are means \pm SEM of duplicates from three independent experiments; a value of 1 was assigned to the cytokine concentration in untreated cells and the relative change after LPS treatment is expressed as -fold induction. Statistics, Student's *t*-test for unpaired values: significant differences LPS vs. control, * = $P < 0.05$; ** = $P < 0.001$. Ctrl,

control; IL-6, interleukin 6; TNF- α , tumor necrosis factor- α .

The persistently low bilirubin, despite excessively elevated LDH, led us to sequence the HO-1 gene; this revealed a homozygous G139V mutation in the patient which was heterozygous in both parents (Figure 1B and 1C). The mutation is located directly in the enzyme's catalytic domain.⁸ Replacements of glycine at this position with larger aliphatic residues cause loss of oxygenase activity but, importantly, also result in gain in peroxidase activity.^{8,9} This mutation was not identified in an additional analysis we performed on 20 HLH patients with unknown genetics, indicating that HO-1 G139V is not a common cause of familial HLH.

We then confirmed the defective enzyme activity by documenting bilirubin synthesis in PBMC. Control cells showed the expected >4-fold heme-induced increase of bilirubin from 18.8 & 23.6 to 95.9 & 84.6 pmol/mg protein/60min following heme treatment. In contrast, patient cells showed a marginal increase from 21.9 & 26.1 to 33.1 & 27.0 pmol/mg protein/60min.

We next assessed HO-1 protein levels in cultured PBMC and found the expected low constitutive and strongly heme-inducible level in healthy controls and in controls with different hemolytic anemias. In contrast, the patient's HO-1 G139V cells showed a strong constitutive HO-1 expression, which did not further increase following hemin treatment (Figure 1D).

The strong constitutive expression of HO-1 G139V with defective normal but gained abnormal peroxidase function was paralleled by increased excretion of peroxidation products in the urine of the patient and, to some degree, in his heterozygous parents and heterozygous brother (Table 2).

[Collapse inline](#) | [View popup](#) | [Download powerpoint](#)
Table 2.

Quantitative determination of ROS metabolites in urine samples.

| | Patient (n=3) | Age matched controls (n=15, mean=30) | Brother (n=1) | Father (n=1) | Brother (n=1) |
|---|------------------|--|------------------|-----------------|------------------|
| Azoxine-ketone (nmol/g Cr) | 580.042 | 294.84 | 363 | 337 | 728 (+13.37.80) |
| Hydroxy-ketone metabolites (U/g Cr) | 4.2-19.2 | 1.2-6.9 | 1.7 | 1.9 | 1.6 (+0.61.50) |
| 8-hydroxy-2'-deoxyguanosine (ng/mg Cr) | 109.125 | 16.3-61.0 | 8.1 | 7.1 | 11.7 (+0.25.65) |
| Protein oxidant (pmol/mg Cr) | 61.5-195 | 35.3-155 | 15.1 | 15.9 | 20.9 (+0.56.24) |

Cr, creatinine.

We then analysed the effect of HO-1 G139V on immunological function in PBMC and showed that exposure to heme led, in contrast to controls, to increased cell death in cytotoxicity assays

Jump To

- Article
- Acknowledgments
- Footnotes
- References
- Figures & Data
- Info & Metrics
- PDF

(Figure 1E), which implies a confounding effect of cell death on HO-1 expression.

Next we determined inflammatory cytokine levels in cultured PBMC in the presence or absence of LPS. In HO-1 G139V cells, the basal IL-6 and TNF- α levels were elevated and responses to LPS were much stronger than in control cells (Figure 1F); this documents a remarkable inflammatory hyperresponsiveness of the patient's PBMC. Considering potential mechanisms of how the mutation may induce hyperinflammation, it is notable that CO, a stoichiometric by-product of heme degradation and bilirubin biosynthesis, also stimulates the interaction between caveolin-1 (cav-1) and TLR4, thus inhibiting TLR4 function.¹⁰ Furthermore, excess heme is known to stimulate TLR4 function.¹¹ Deregulated TLR4 function may therefore play a role in the pathogenesis of hyperinflammation in this patient.

With the broader availability of whole exome sequencing, we searched for other mutations which could plausibly explain the phenotype of the patient. *De novo* homozygous mutations in the SPON2 and IBTK genes, which bind LPS and regulate B-cell function, respectively, were detected in the patient and were found to be heterozygous in the parents. These mutations may contribute to the phenotype. Additionally, in the absence of whole genome sequencing, significant non-exonic mutations might have been missed.

It is interesting to compare the features of this patient carrying the G139V mutation with those of two children with absent HO-1 expression.^{5,6,12,13} Both null-mutations and G139V cause hemolytic anemia with almost completely abolished bilirubin synthesis. Interestingly, in HO-1^{-/-} mice splenic macrophages are eliminated by the toxic effect of intracellular heme, resulting in splenic fibrosis and intravascular hemolysis.¹⁴ Consistent with this phenotype in mice, asplenia occurred in previously described patients with HO-1 null-mutations. In contrast, the G139V-mutated patient had splenomegaly. Both the null-mutations and G139V resulted in signs of endothelial damage; though this was much more severe in the patients with null-mutations. Abnormal red cell morphology was also observed in both types of mutation; however, the patients with null-mutants suffered the most severe transfusion-dependent hemolytic anemia, whilst

Jump To

- [Article](#)
- [Acknowledgments](#)
- [Footnotes](#)
- [References](#)
- [Figures & Data](#)
- [Info & Metrics](#)
- [PDF](#)

the G139V-patient showed only transient severe anemia and mild anemia subsequently. In remarkable contrast to the patient with the deletion mutant, iron supplementation and red-cell transfusion in the G139V-patient were associated with the development of life-threatening inflammation. Additionally, iron depletion by calculated phlebotomy was associated with continuous improvement of anemia and long-term remission of HLH. These important differences can be explained by the gain of abnormal peroxidase function of the strong, constitutive expression of HO-1 G139V, which was predicted by previous biochemical analyses^{8,9} and confirmed by the increased excretion of urinary peroxidation products in the patient's urine. Despite the fact that we cannot provide a genetic model for this disease, it is intriguing that this abnormal biochemical function distinguishes this novel clinical entity from that caused by HO-1 null-mutations, although there is overlap caused by loss of HO-1 function in both types of mutants.

These clinical and functional observations confirm the pathogenetic role of the iron and heme inducible mutated HO-1 protein in this disorder. In principle, one may consider complementing this treatment with other antioxidant or iron chelating medication; this has not been done here due to the current stable condition of the patient without further intervention.

The case reported here demonstrates the clinical importance of HO-1 function in oxidative stress defense and in the regulation of macrophage-dependent inflammatory responses.

Acknowledgments

We acknowledge Viktoria Jeney for expert technical assistance and The Dietmar Hopp Stiftung and The German Ministry of Science and Technology (E-rare) for financial support. JB is supported by the Hungarian Academy of Sciences (11003).

Footnotes

- ¹* senior authors
- This study is dedicated to the late Hermann Heimpel who significantly contributed to this study.
- Funding: JB is supported by the Hungarian Academy of Sciences (11003).

Jump To

- Article
- Acknowledgments
- Footnotes
- References
- Figures & Data
- Info & Metrics
- PDF

- Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

Copyright© Ferrata Storti Foundation

References

1. Janka GE, Schneider EM. Modern management of children with haemophagocytic lymphohistiocytosis. *Br J Haematol*. 2004;**124**(1):4–14. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
2. Menasche G, Feldmann J, Fischer A, de Saint Basile G. Primary hemophagocytic syndromes point to a direct link between lymphocyte cytotoxicity and homeostasis. *Immunol Rev*. 2005;**203**:165–179. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
3. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol*. 1997;**37**:517–554. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
4. Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol*. 2010;**50**:323–354. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
5. Yachie A, Niida Y, Wada T, et al. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest*. 1999;**103**(1):129–135. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
6. Radhakrishnan N, Yadav SP, Sachdeva A, et al. Human heme oxygenase-1 deficiency presenting with hemolysis, nephritis, and asplenia. *J Pediatr Hematol Oncol*. 2011;**33**(1):74–78. [CrossRef](#) [Medline](#) [Google Scholar](#)
7. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;**48**(2):124–131. [CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)
8. Liu Y, Koenigs Lightning L, Huang H, et al. Replacement of the distal glycine 139 transforms human heme oxygenase-1 into a peroxidase. *J Biol Chem*. 2000;**275**(44):34501–34507. [Abstract/FREE Full Text](#) [Google Scholar](#)
9. Lad L, Koshkin A, de Montellano PR, Poulos TL. Crystal structures of the G139A, G139A-NO and G143H mutants of human heme oxygenase-1. A finely tuned hydrogen-bonding network controls oxygenase versus peroxidase activity. *J Biol Inorg Chem*. 2005;**10**(2):138–146. [CrossRef](#) [Medline](#) [Google Scholar](#)
10. Wang XM, Kim HP, Nakahira K, Ryter SW, Choi AM. The heme oxygenase-1/carbon monoxide pathway suppresses TLR4 signaling by regulating the interaction of TLR4 with caveolin-1. *J Immunol*. 2009;**182**(6):3809–3818. [Abstract/FREE Full Text](#) [Google Scholar](#)
11. Vinchi F, Costa da Silva M, Ingoglia G, et al. Hemopexin therapy reverts heme-induced

Jump To

- Article
- Acknowledgments
- Footnotes
- References
- Figures & Data
- Info & Metrics
- PDF

proinflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease. *Blood*. 2016;**127**(4):473–486

[Abstract/FREE Full Text](#) [Google Scholar](#)

12. J Kawashima A, Oda Y, Yachie A, Koizumi S, Nakanishi I. Heme oxygenase-1 deficiency: the first autopsy case. *Hum Pathol*. 2002;**33**(1): 125–130.

[CrossRef](#) [Medline](#) [Web of Science](#) [Google Scholar](#)

13. J Saikawa Y, Kaneda H, Yue L, et al. Structural evidence of genomic exon-deletion mediated by Alu-Alu recombination in a human case with heme oxygenase-1 deficiency. *Hum Mutat*. 2000;**16**(2):178–179. [Medline](#) [Google Scholar](#)

14. J Kovtunovych G, Eckhaus MA, Ghosh MC, Ollivierre-Wilson H, Rouault TA. Dysfunction of the heme recycling system in heme oxygenase 1-deficient mice: effects on macrophage viability and tissue iron distribution. *Blood*. 2010;**116**(26):6054–6062.

[Abstract/FREE Full Text](#) [Google Scholar](#)

Jump To

[Article](#)
[Acknowledgments](#)
[Footnotes](#)
[References](#)
[Figures & Data](#)
[Info & Metrics](#)
[PDF](#)

[Previous](#)

[Next](#)

[Back to top](#)

Navigate

[Home](#)
[Current issue](#)
[Ahead of print](#)
[Archive](#)
[Info for](#)
[Authors](#)
[Reviewers](#)
[Advertisers](#)
[Subscribers](#)
[About us](#)
[About](#)
[Haematologica](#)
[Editorial Board](#)
[Our policies](#)
[About EHA](#)
[CME](#)

For Authors

[Author guidelines](#)
[Submit Manuscript](#)
[Track Manuscript](#)

[For Reviewers](#)
[Reviewer Guidelines](#)
[Access Your Profile](#)
[Access Your Tasks](#)
[2014 reviewers](#)

For Advertisers

[Information For Advertising](#)

[Education](#)
[Review Articles](#)
[Guideline Articles](#)
[EHA Education Book](#)
[EHA Education Tools](#)
[CME](#)

More

[Rights & Permissions](#)
[Advertising](#)
[Alerts](#)
[Feedback](#)
[Contact](#)
[App](#)

[EHA](#)
[About EHA](#)
[Become a member of EHA](#)
[EHA events](#)
[EHA education tools](#)
[EHA Corporate Sponsors](#)

[Copyright © 2017 by the Ferrata Storti Foundation](#)

ISSN 0390-6078 print

ISSN 1592-8721 online

Powered by