Blood Cells, Molecules and Diseases 77 (2019) 137–141

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Short Communication

The Growth Differentiation Factor-15 (GDF-15) levels are increased in patients with compound heterozygous sickle cell and beta-thalassemia (HbS/β^{thal}), correlate with markers of hemolysis, iron burden, coagulation, endothelial dysfunction and pulmonary hypertension

Katerina Larissi[a,b,1], Marianna Politou[c,1], Alexandra Margeli[a], Christos Poziopoulos[a], Pagona Flevari[b], Evangelos Terpos[d], Ioannis Papassotiriou[a], Ersi Voskaridou[b,*]

[a] Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Greece
[b] Thalassemia and Sickle Cell Disease Center, "Laikon" University General Hospital, Greece
[c] Laboratory of Hematology and Blood Bank Unit, "Aretaieio" Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece
[d] Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

ARTICLE INFO

Editor: Mohandas Narla

Keywords:
Sickle Cell Disease
βthalassaemia
Endotheial dysfunction
GDF-15

ABSTRACT

The clinical manifestations of Sickle Cell Disease (SCD) include episodes of vascular occlusion, chronic hemolytic anemia and frequent infections. GDF-15, a multifactorial cytokine, is a member of the transforming growth factor- superfamily. Expression of the GDF-15 gene in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury, while high levels of serum GDF-15 associate with ineffective erythropoiesis and may reflect a certain type of bone marrow stress or erythroblast apoptosis. In this context we aimed to evaluate GDF-15 levels in 89 patients with HbS/β^{thal} at steady phase and in 20 apparently healthy individuals, and correlate with clinical features of the disease and markers of hemolysis, iron burden, inflammation, coagulation and endothelial dysfunction. We found that: GDF-15 levels were elevated in patients with HbS/β^{thal} compared to controls (1980.7 ± 159.8 vs 665.4 ± 50.9 pg/mL, $p < 0.0001$) and correlated significantly with LDH ($p < 0.001$), Hepcidin-25/Ferritin molar ratio ($p = 0.002$), vWF:antigen ($p < 0.05$), HbA% ($p < 0.001$) and Mean Pulmonary Artery Pressure ($p < 0.001$). These findings demonstrate for first time an important multifactorial role of GDF-15 in patients with HbS/β^{thal}, however, prior to its clinical usefulness, this biomarker must undergo through rigorous validation in multiple cohorts.

1. Introduction

Sickle Cell Disease (SCD) is a hereditary haemoglobinopathy, characterized by haemolysis and microvascular vaso-occlusion which causes a severe clinical condition with complex pathophysiology and a marked phenotypic variability. The latter reflects the interplay of a large number of factors which include leukocyte dysfunction, platelet interactions with endothelial cells, increased levels of circulating pro-inflammatory cytokines, oxidative stress, endothelial damage, decreased availability of nitric oxide, activation of haemostasis and enhanced microvascular thrombosis [1]. Laboratory evidence for these processes are the altered levels of an array of indices, such as the levels of hemoglobin (Hb), reticulocytes and GDF-15 as indices of erythropoiesis, the levels of LDH and reticulocytes as markers of haemolysis, the levels of ADAMTS-13 and vWF denoting endothelial activation, hs-CRP as a marker of inflammation, and D-Dimers as an index of activated coagulation [2,3].

Hydroxycarbamide (hydroxyurea, HU) has been safely and efficiently used over the past 25 years SCD in order to reduce the frequency and severity of the acute vaso-occlusive events (pain crises, acute chest syndrome, splenic sequestration, acute anemia) as well as the impact of several chronic complications [4,5].

Although many from the above mentioned indices have been individually assessed in SCD patients, no study so far has extended the analysis of all the above markers in a cohort of HbS/β^{thal} patients in steady state i.e. in the absence of vaso-occlusive events over a six

* Corresponding author at: Thalassemia and Sickle Cell Disease Center, "Laiko" General Hospital, 16 Sevastoupoleos Street, GR 11526 Athens, Greece.
E-mail address: ersi.voskaridou@gmail.com (E. Voskaridou).
[1] The authors have equally contributed.

https://doi.org/10.1016/j.bcmd.2019.04.011
Received 25 February 2019; Received in revised form 22 April 2019; Accepted 22 April 2019
Available online 23 April 2019
1079-9796/

months period prior to sampling taking into account HU treatment.

GDF-15, a multifactorial cytokine, is a member of the transforming growth factor- superfamily Expression of the GDF-15 gene in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury, while high levels of serum GDF-15 associate with ineffective erythropoiesis and may reflect a certain type of bone marrow stress or erythroblast apoptosis [6–9].

Although elevated GDF-15 levels are documented both in thalassemia (major and intermedia), and in SCD patients data on the role of GDF-15 in compound heterozygotes HbS/β^{thal} are sparse [10–12].

The aim of the study was to assess possible correlations of GDF-15 with the above mentioned markers as well as with disease complications.

2. Subjects and methods

The study was approved by the Institutional Review Committee for the protection of human subjects at 'Laikon' General Hospital and conducted in agreement with the Helsinki declaration of 2000. Written informed consent from all patients and controls was obtained prior to patient blood sampling.

Eighty-nine (34 males and 55 females) adult Caucasian HbS/β^{thal} patients with no history of blood transfusion and crises in the previous six months were enrolled. Median age was 47.9 years (range: 24–81). History of clinical complications such as vaso-occlusive crises (VOC), acute chest syndrome, venous thrombosis, arterial thrombosis including stroke, and, avascular necrosis were recorded. Pulmonary artery pressure was assessed through Doppler echocardiography and calculation of the tricuspid regurgitant jet velocity (TRV). The patients were divided into two subgroups: those being treated regularly with hydroxycarbamide (group A, 49 patients) and those not receiving this agent (group B, 40 patients). The indications of HU treatment were three or more painful crises per year or any complications such as acute chest syndrome (ACS), acute or silent stroke.

Patients had the following genotypes: HbS/IVSI-6 (six patients), HbS/IVSI-110 (thirty one patients), HbS/-87 C > G (one patient), HbS/δβSic (one patient), HbS/CD39 (eleven patients), HbS/IVSI-1 (six patients), HbS/IVSII-745 (three patients), HbS/FSC-6 (two patients), HbS/IVSI-5 (one patient), HbS/IVSII-1 (one patient). For twenty five HbS/βthal patients genotype was missing. The distribution of the mutations in the two groups of patients was as follows: 14/42 in the HU(+) group had β^o mutations the rest had β^+ mutations while in the HU(−) group 4/22 had β^o mutations and 18/22 had β^+ mutations.

Twenty apparently healthy HbA/HbA individuals served as controls.

The laboratory investigation included WBC, reticulocytes, blood chemistry parameters including LDH and uric acid. Circulating levels of high-sensitivity C-reactive protein (hs-CRP) were measured by means of immunonephelometric techniques using the BN Prospec nephelometer (Dade Behring; Siemens Healthcare Diagnostics, Liederbach, Germany). Ferritin was quantitatively determined using the Roche E411 Cobas immunoassay analyzer (Roche Diagnostics, Mannheim, Germany), using an electrochemiluminescence technique. vWF:antigen (vWF:Ag) (INNOVANCE® VWF Ac) and D-Dimers (INNOVANCE® D-Dimer) levels were measured in automated analyzer (SYSMEX CA-1500, Siemens Healthcare GmbH, Munich). Circulating levels of ADAMTS-13 antigen, were measured by means of immunoenzymatic technique (R&D Systems, Minneapolis, MN). Hepcidin-25 levels were measured by an enzyme-linked immunosorbent assay (Intrinsic LifeSciences, La Jolla, CA, USA). GDF-15 levels were measured with an automated immunoassay on Roche Cobas electrochemiluminescence systems (Roche Diagnostics International Ltd., Rotkreuz, Switzerland).

2.1. Statistical analyses

Data are presented as mean ± SD, and the level of statistical significance was considered at $p < 0.05$. All the statistical procedures were performed using the STATGRAFICS PLUS version 5.1 for Windows program (Graphic Software System). We used the standardized skewness and standardized kurtosis, to determine whether the sample comes from a normal distribution. Values of these statistics outside the range of −2 to +2 indicate significant departures from normality, which would tend to invalidate many of the statistical procedures normally applied to this data. These values integrated automatically from the program, indicating which parameters needed to transform in either log or reciprocal or square root. These transformations were used then for correlations between parameters.

3. Results

From the 89 patients with HbS/β^{thal} 15 had experienced acute chest syndrome, 11 vascular thrombosis 10 stroke and 29 avascular necrosis while 28 patients had pulmonary pressure > 25 mmHg.

LDH, ferritin, hs-CRP, vWF, D-Dimers, and GDF-15 were elevated in HbS/β^{thal} patients compared to controls. Conversely, the levels of ADAMTS-13 and hepcidin-25 and the hepcidin25/ferritin molar ratio were lower in the SCD patients compared to controls ($p < 0.001$, $p < 0.01$ and $p < 0.001$ respectively). The levels of hepcidin-25 and GDF-15 were significantly higher in the group A patients compared to those of group B ($p < 0.01$), while the levels of hs-CRP, vWF:Ag and ADAMTS-13 and D-Dimers failed to show any meaningful differences between the two groups (Table 1). GDF-15 levels did not differ between HbS/β^othal and HbS/β^+thal patients.

Hs-CRP levels correlated positively with those of vWF ($r = 0.360$, $p < 0{,}001$) and ADAMTS-13 ($r = 0.382$, $p = 0{,}002$). Ferritin levels correlated positively with hs-CRP ($r = 0.360$, $p < 0.001$), vWF ($r = 0.317$, $p = 0.004$), D-Dimers ($r = 0.344$, $p = 0.002$) and GDF-15 ($r = 0.420$, $p < 0.001$) as well as with all markers of haemolysis LDH ($r = 0.328$ $p < 0.001$). Almost all (92%) patients had abnormal D-

Table 1
Hematologic and blood chemistry parameters in patients with HbS/β^{thal} and healthy controls.

	HbS/β^{thal} (HU+) N = 49	HbS/β^{thal} (HU−) N = 40	HbS/β^{thal} (ALL) N = 89	Controls N = 20
Hematologic parameters				
Hb (g/L)	94 ± 13	98 ± 17	96 ± 15	125–160[d]
Hb A (%)	6.6 ± 6.0	9.9 ± 9.1	8.3 ± 7.9	> 96.5[d]
Hb F(%)	20.4 ± 9.2	6.7 ± 5.9[c]	14.2 ± 10.4	< 1.0[d]
MCV (fL)	85.5 ± 3.1	68.1 ± 1.8[c]	77.7 ± 12.3	85.0–100.5
Reticulocytes (%)	5.4 ± 2.7	4.6 ± 2.8	5.0 ± 2.8	0.5–1.2[d]
Blood chemistry parameters				
Uric acid (mg/dL)	5.4 ± 1.3	5.2 ± 1.7	5.3 ± 1.5	4.8 ± 1.3
LDH (U/L)	657 ± 213	602 ± 240	632 ± 226	375 ± 65[d]
Ferritin (μg/L)	422 ± 355	351 ± 699[c]	447 ± 367	42 ± 22[d]
Special chemistry parameters				
hs-CRP (mg/L)	7.5 ± 5.7	6.2 ± 4.8	6.9 ± 5.3	0.43 ± 0.10[d]
vWF:antigen (IU/dL)	174 ± 72	168 ± 74	171 ± 78	85 ± 22[d]
D-Dimers (pg/mL)	929 ± 255	983 ± 236	956 ± 245	340 ± 187[d]
GDF-15 (pg/mL)	2478 ± 222	1520 ± 204	1980 ± 159	665 ± 50[c]
Hepcidin-25 (ng/mL)	68.9 ± 9.1	46.1 ± 8.1[b]	57.2 ± 6.2	67.3 ± 4.5[d]
Hepcidin-25/Ferritin (molar ratio)	31 ± 3	53 ± 10[a]	43 ± 5	320 ± 38[d]

Significant differences: patients with HbS/β^{thal} HU+ vs HU−, [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$, significant differences: patients with HbS/B^{thal} vs controls [d]$p < 0.05$-$p < 0.001$.

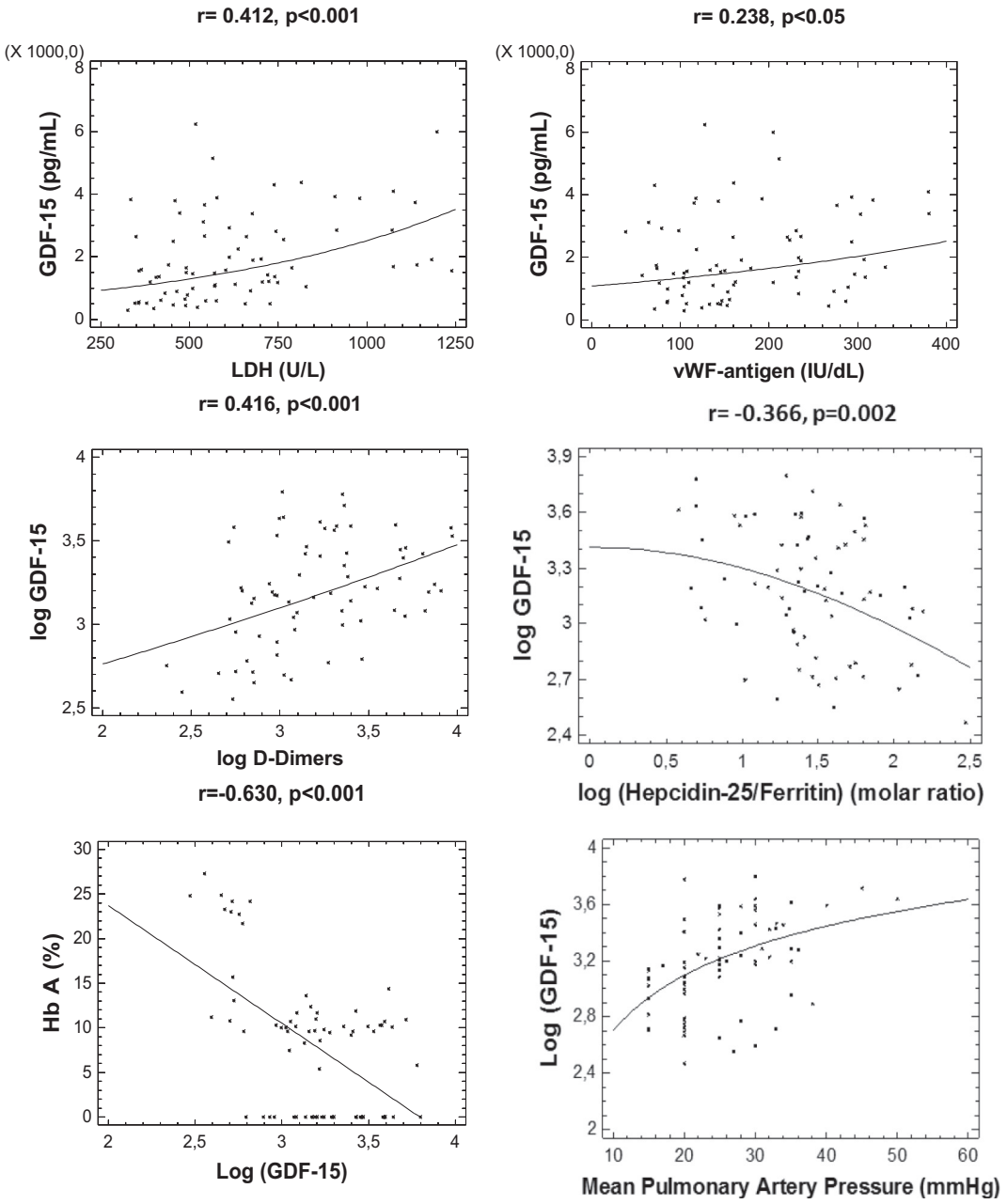

Fig. 1. Correlation between GDF-15 levels with LDH (a), vWF:antigen (b), D-Dimers (c), concentrations and Hepcidin-25/Ferritin molar ratio (d), HbA% (e), Pulmonary pressure (f).

Dimers levels.

GDF-15 levels correlated significantly with markers of erythropoiesis, such as Hb levels and reticulocytes ($r = -0.424$, $p < 0.01$; $r = 0.510$, $p < 0.001$, respectively), with LDH ($r = 0.412$, $p < 0.001$), uric acid ($r = 0.321$, $p = 0.005$), as well as with the vWF:Ag ($r = 0.238$, $p < 0.05$). GDF-15 levels and the hepcidin-25/ferritin molar ratio showed a significant negative correlation ($r = -0.366$ $p = 0.002$), while no correlation was found between GDF-15 and hs-CRP ($p > 0.65$) (Fig. 1).

We assessed HbA% as the most relevant marker of the β-thal genotype and it correlated with GDF-15 levels {logGDF-15 ($r = -0.636$, $p < 0.001$)} Fig. 1e. There was also a weak correlation of HbA% with ferritin ($r = -0.375$, $p < 0.001$) and D-Dimers ($r = -0.359$, $p < 0.001$).

In terms of clinical complications vascular thrombosis correlated negatively with HbF = −0.329, p = 0.008, while LDH correlated positively with VOC ($r = 0.356$, $p = 0.02$).

Pulmonary Pressure correlated positively with retics $r = 0.376$, $p = 0.004$ and GDF-15($r = 0.460$ $p < 0.001$) (Fig. 1f). Patients with pulmonary pressure > 25 mmHg had higher GFD-15 levels ($p < 0.001$).

4. Discussion

The pathophysiology of SCD includes haemolytic anemia, vaso-occlusive episodes, endothelial injury and a low grade non septic inflammation. The laboratory evidence for these processes is reflected by the altered levels of an array of indices, which have been listed in the introduction. We evaluated the same factors in 89 HbS/β^{thal} patients in steady state. Further on, the patients were also categorized according to their treatment or no treatment with hydroxycarbamide.

Our results indicate that all processes known to occur in active SC

disease are detectable also in the "steady state" of this condition. More specifically in our patients a sustained endothelial activation as evidenced by elevated serum levels of vWF:Ag levels compared to controls was recorded. Our data are in agreement with reports of increased vWF:Ag levels at steady state of SCD with further elevation during vaso-occlusive crisis (VOC) [3]. ADAMTS-13, the main regulator of vWF homeostasis was decreased in our HbS/β^{thal} patients compared to controls, although normal levels of ADAMTS13 antigen were reported from other groups [13]. It has been shown that ADAMTS-13 cleavage of vWF is inhibited by neutrophil oxidants [14] and extracellular hemoglobin [15] while several inflammatory cytokines significantly inhibit its synthesis and secretion [16].

We could hypothesize that inflammation along with reduced circulating levels of ADAMTS-13 could lead to the increased levels of vWF. Both vWF and ADAMTS-13 levels correlated positively with inflammation (hs-CRP). Thus our patients seem to experience chronic systemic inflammation even at steady state.

We have also found a positive correlation of ferritin with the vWF factor and although ferritin, as an acute phase molecule may not accurately predict iron stores, in the SCD setting, we could consider that endothelial dysfunction may be a reflection of inflammatory pathways and oxidative stress. Furthermore, intravascular haemolysis that occurs in SCD as evidenced by increased LDH levels may also trigger vascular inflammatory process as assessed by the correlation of LDH with ferritin. Inflammation in this cohort may also enhance coagulation, a notion based on positive correlation of elevated D-Dimers levels with ferritin.

Almost all our patients had abnormal D-Dimers levels, indicating a hypercoagulable state in SCD patients even at the steady state while HU treatment does not seem to alter that constant coagulation activation. Our results are in agreement with previous reports that have shown alterations in the haemostatic pathway that may explain the thrombotic complications observed in SCD [2].

In the literature, the contribution of hydroxyurea treatment to the reduction of soluble levels of endothelial adhesion and inflammatory molecules has been contradictory [5,17]. However, we were unable to demonstrate statistically significant differences in levels of markers of endothelial dysfunction and inflammation between group A and B of our patients probably due to an inherent selection bias since patients with a more severe form of the disease are treated with HU.

In terms of iron and erythropoiesis interplay we have found that HbS/βthal patients had higher GFF-15, ferritin and lower hepcidin/ferritin ratio compared to controls while patients receiving hydroxyurea had statistically significant increased ferritin, hepcidin-25 and GDF-15 compared to untreated patients.

Intense erythropoietic activity and anemia reduces hepcidin transcription while iron overload and inflammation induces it [18]. GDF-15, an erythroblast-expressed factor, contributes to the incomplete suppression of hepcidin in pathological situations with ineffective erythropoiesis [10] but is not likely involved in the physiological suppression of hepcidin for normal erythropoiesis [19]. SCD patients exhibit increased erythropoiesis but only in severe cases ineffective erythropoiesis maybe found [12].

In our study, patients had statistically significant elevated GDF-15 levels compared to controls which are consistent with the findings in the literature for both pediatric and adult patients express higher levels of GDF-15 while data for HbS/βthal patients are limited to 13 patients [12]. The elevated GDF-15 levels found in our cohort can be partly attributed to ineffective erythropoiesis due to β-thal component of our patients but it may be also partially related to tissue ischemia. It is well established that GDF-15 is not only produced by the erythroblasts and its expression in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury [6–8].

The higher GDF-15 levels measured in patients treated with hudroxycarbamide could be attributed to the higher proportion of HbS/β^{o}thal patients in this group of patients in which the drug has only partially suppressed ineffective erythropoiesis and the ensuing negative vascular effects. When we assessed HbA% as the most relevant marker of the β thal genotype we found that HbA% correlates with GDF-15 levels and thus genotype may be a determinant of GDF-15 levels and of the extend of ineffective erythropoiesis.

Hepcidin-25 levels were significantly lower in our patients compared to controls; similar to findings reported by Kroot et al. suggesting that GDF-15 could mediate hepcidin suppression and enhance iron accumulation [20]. Hepcidin/ferritin ratio (a marker of appropriateness hepcidin produced for the iron burden), was significantly lower for patients. However, since inflammation may mediate ferritin's increase the suitability of the ratio for evaluating adequacy of hepcidin in response to iron loading is questionable.

It has been shown that GDF-15 levels are related to hemolysis and iron overload in SCD patients but in our study apart from confirming these findings we also found that GDF-15 levels correlated positively with markers of endothelial dysfunction (vWF) and coagulation (D-Dimers), implying that tissue ischemia may also increase its levels. Furthermore, ineffective erythropoiesis may promote coagulation by enhancing the release of red cells with prothrombotic potential in the circulation.

In terms of clinical manifestations VOC correlated with LDH and vascular thrombosis correlated negatively with HbF% emphasising the role of sickling and peripheral hemolysis in the clinical picture of the disease.

It is also of interest that in our cohort of patients GDF-15 levels correlated with pulmonary pressure. It is well documented that GDF-15 is strongly expressed in the vascular compartment of patients with pulmonary hypertension and hypoxia and stress is a potent stimulator of its expression in pulmonary endothelial cells [21]. In a previous study GDF-15 was not different between patients with or without pulmonary hypertension and the authors attributed this finding to the fact that the pathophysiology of PH in SCD differs from that in thalassemia [12].

Pulmonary hypertension, is common both in patients with thalassemia (major and intermedia) and Sickle Cell Disease [22,23].

The pathophysiologic mechanism that underlies PH in these clinical entities seems to be distinct. In thalassemia syndromes PH is thought to be the result of chronic hemolysis, iron overload due to transfusion therapy, hypercoagulability, and changes of circulating cells as a consequence of splenectomy while in SCD anemia and sickling seem to play a role [24–26].

In our group of patients both mechanisms may contribute to the manifestation of pulmonary hypertension.

5. Conclusion

Our study highlights the interplay among endothelial dysfunction, tissue ischemia, ineffective erythropoiesis and inflammation in HbS/β-thal patients even at a prolonged steady state (at least 6 months without vaso-occlusive events).

Patients with HbS/β-thal have a significant degree of endothelial dysfunction (as assessed by increased vWF) and of activation of coagulation (increased D-Dimers levels). GDF-15 levels are related to hemolysis, hypercoagulation and endothelial dysfunction and may provide utility in identifying a subgroup of higher risk patients.

Our findings demonstrate for first time an important multifactorial role of GDF-15 in patients with HbS/β^{thal}, strengthening the importance of GDF-15 in the pathophysiology of the disease. Its role may be direct on endothelial dysfunction through tissue ischemia and/or indirect with endothelial dysfunction and coagulation mediated by ineffective erythropoiesis and hemolysis (i.e. release of red cell contents by damaged erythrocytes, and inflammation). However, prior to its clinical usefulness, this biomarker must undergo through rigorous validation in multiple cohorts.

It remains to be seen whether new therapeutic options for SCD, now in the pipeline, which increase fetal hemoglobin expression, inhibit red cell sickling, clotting or cell to cell interactions could intervene and alter that interplay.

Acknowledgements

KL performed the research and wrote the paper, MP designed the study, performed the research and wrote the paper, AM performed the research and analyzed the data, CP, analyzed the data, ET analyzed the data and critically revised the paper, PF was responsible for the clinical care of the patients, IP designed the study performed the research, analyzed the data and wrote the paper, EV was responsible for the clinical care of the patients. Research Funding was received from Athens University to Professor E.T. (ELKE 11217). The funding source played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

[1] G.J. Kato, R.P. Hebbel, M.H. Steinberg, M.T. Gladwin, Vasculopathy in sickle cell disease: biology, pathophysiology, genetics, translational medicine and new research directions, Am. J. Hematol. 84 (9) (2009) 618–625.

[2] K.I. Ataga, J.E. Brittain, P. Desai, R. May, S. Jones, J. Delaney, et al., Association of coagulation activation with clinical complications in sickle cell disease. Covas DT, ed, PLoS One 7 (1) (2012) e29786.

[3] J. Chen, W.E. Hobbs, J. Le, P.J. Lenting, P.G. de Groot, J.A. López, The rate of hemolysis in sickle cell disease correlates with the quantity of active von Willebrand factor in the plasma, Blood 117 (13) (2011) 3680–3683.

[4] Charache, M. L. Terrin, R. D. Moore, G. J. Dover, F. B. Barton, S. V. Eckert, R. P. McMahon, D. R. Bonds. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N. Engl. J. Med. (1995) May 18; 332(20) pp: 1317–1322.

[5] E. Voskaridou, V. Kalotychou, D. Loukopoulos, Clinical and laboratory effects of long term administration of hydroxyurea to patients with sickle cell/β-thalassaemia, Br. J. Haematol. 89 (1995) 479–484.

[6] A.R. Bauskin, D.A. Brown, T. Kuffner, H. Johnen, X.W. Luo, M. Hunter, et al., Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer, Cancer Res. 66 (2006) 4983–4986.

[7] T. Ago, J. Sadoshima, GDF15, a cardioprotective TGF-beta superfamily protein, Circ. Res. 98 (2006) 294–297.

[8] T. Kempf, M. Eden, J. Strelau, M. Naguib, C. Willenbockel, J. Tongers, et al., The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury, Circ. Res. 98 (3) (2006) 351–360 Feb 17.

[9] T. Tanno, P. Noel, J.L. Miller, Growth differentiation factor 15 in erythroid health and disease, Curr. Opin. Hematol. 17 (3) (2010) 184–190, https://doi.org/10.1097/MOH.0b013e328337b52f May.

[10] T. Tanno, V.N. Bhanu, P.A. Oneal, S. Goh, P. Staker, Y.T. Lee, et al., High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin, Nat. Med. 13 (9) (2007) 1096–1101 Sep.

[11] K.M. Musallam, A.T. Taher, L. Duca, C. Cesaretti, R. Halawi, M.D. Cappellini, Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with β thalassemia intermedia, Curr. Opin. Hematol. 17 (3) (2010) 184–190, https://doi.org/10.1097/MOH.0b013e328337b52f (May).

[12] T.A. Gawad, A. MoneamAdly, E.R. Ismail, Y. WagihDarwish, M.A. Zedan, Growth differentiation factor-15 in young sickle cell disease patients: relation to hemolysis, iron overload and vascular complications, Blood Cells Mol. Dis. 53 (4) (2014) 189–193 Dec.

[13] A. Al-Awadhi, A. Adekile, R. Marouf, Evaluation of von Willebrand factor and ADAMTS-13 antigen and activity levels in sickle cell disease patients in Kuwait, J. Thromb. Thrombolysis 43 (2017) 117.

[14] J. Chen, X. Fu, Y. Wang, L. Minhua, B. McMullen, J. Kulman, et al., Oxidative modification of von Willebrand factor by neutrophil oxidants inhibits its cleavage by ADAMTS13, Blood 115 (3) (2010) 706–712.

[15] Z. Zhou, H. Hyojeong, M.A. Cruz, J.A. López, J.F. Dong, P. Guchhait, Haemoglobin blocks von Willebrand factor proteolysis by ADAMTS-13: a mechanism associated with sickle cell disease, J. Thromb. Haemost. 101 (6) (2009) 1070–1077 Jun.

[16] W. Cao, M. Niiya, X. Zheng, D. Shang, X.L. Zheng, Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells, J. Thromb. Haemost. 6 (7) (2008) 1233–1235.

[17] R.R. Penkert, J.L. Hurwitz, P. Thomas, J. Rosch, J. Dowdy, Y. Sun, et al., Inflammatory molecule reduction with hydroxyurea therapy in children with sickle cell anemia, Haematologica 103 (2) (2018) e50–e54.

[18] M.S. Karafin, K.L. Koch, A.B. Rankin, D. Nischik, G. Rahhal, P. Simpson, et al., Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease, Blood Cells Mol. Dis. 55 (4) (2015) 304–307.

[19] K. Junya, C. Mizumoto, H. Kawabata, H. Tsuchida, N. Tomosugi, K. Matsuo, et al., Serum hepcidin level and erythropoietic activity after hematopoietic stem cell transplantation, Haematologica 93 (10) (2008) 1550–1554 Oct.

[20] J.J. Kroot, C.M. Laarakkers, E.H. Kemna, B.J. Biemond, D.W. Swinkels, Regulation of serum hepcidin levels in sickle cell disease, Haematologica 94 (6) (2009) 885–887, https://doi.org/10.3324/haematol.2008.003152.

[21] N. Nickel, H. Golpon, M. Greer, L. Knudsen, K. Olsson, V. Westerkamp, et al., The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension, Eur. Respir. J. 39 (3) (2012) 589–596 Mar.

[22] D.R. Fraidenburg, R.F. Machado, Pulmonary hypertension associated with thalassemia syndromes, Ann. N. Y. Acad. Sci. 1368 (1) (2016) 127–139 Mar.

[23] M.T. Gladwin, V. Sachdev, M.L. Jison, Y. Shizukuda, J.F. Plehn, K. Minter, et al., Pulmonary hypertension as a risk factor for death in patients with sickle cell disease, N. Engl. J. Med. 350 (9) (2004) 886–895 Feb 26.

[24] F. Parent, D. Bachir, J. Inamo, F. Lionnet, F. Driss, G. Loko, et al., A hemodynamic study of pulmonary hypertension in sickle cell disease, N. Engl. J. Med. 365 (1) (2011) 44–53 Jul 7.

[25] A.M. Yates, V.M. Joshi, B. Aygun, J. Moen, M.P. Smeltzer, D. Govindaswamy, et al., Elevated tricuspid regurgitation velocity in congenital hemolytic anemias: prevalence and laboratory correlates, Pediatr. Blood Cancer (2019) e27717(Mar 25) [Epub ahead of print].

[26] V.P. Bilan, F. Schneider, E.M. Novelli, E.E. Kelley, S. Shiva, M.T. Gladwin, et al., Experimental intravascular hemolysis induces hemodynamic and pathological pulmonary hypertension: association with accelerated purine metabolism, Pulm Circ 8 (3) (2018) Jul-Sep. (2045894018791557).