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Angiogenic profiling in HELLP syndrome cases with or without hypertension and proteinuria

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ABSTRACT

Objectives: Angiogenic profiling with the use of sFlt-1/PlGF ratio (soluble fms-like tyrosine kinase-1/placental growth factor) can be helpful to characterize women with signs of impending preeclampsia (PE). However, little is known about the angiogenic profile of pregnancies complicated by HELLP syndrome. The aim of this study was to examine the relationship of angiogenic profiles in cases of HELLP syndrome with and without classical signs of preeclampsia.

Study design: The angiogenic profile of pregnant women with singleton gestation and isolated PE (group 1), PE associated with HELLP syndrome (group 2), and isolated HELLP syndrome (group 3) from 01/2011 to 03/2018, were compared. To overcome gestational age dependent angiogenic behavior, cases (group 3) were matched 1:2 with cases from group 1 and 2. Matching criteria was gestational age (±1 week). PE and HELLP syndrome were defined according to the international Society for the Study of Hypertension in Pregnancy (ISSHP) statement 2014.

Results: During the observational period, 244 women could be included in the study. Of those, 237 (97.1%) were diagnosed with PE. In 42 cases (17.2%) PE was associated with HELLP syndrome while 7 (2.9%) patients were diagnosed with isolated HELLP syndrome. Angiogenic profiles in terms of sFlt-1/PlGF ratios differed significantly between the three groups, showing highest levels in group 2 (PE/HELLP) while cases with isolated HELLP demonstrated the lowest ratios and sFlt-1 values ($p = 0.01$).

Conclusion: We conclude that isolated HELLP syndrome is rare and seems to be a particular entity expressing a different angiogenic behaviour compared to classical PE or PE associated with HELLP syndrome.

Introduction

Preeclampsia and HELLP (haemolysis, elevated liver enzyme, low platelet) syndrome are among the leading causes of maternal morbidity and mortality in developed countries [1]. Preeclampsia is defined as new onset hypertension after 20 weeks of gestation and proteinuria or new onset hypertension and maternal organ dysfunction or signs of utero-placental dysfunction with or without proteinuria [2]. Signs of systemic dysfunction are neurological symptoms such as headache or visual disturbances, thrombocytopenia or abnormal liver or renal function resulting from mild to severe microangiopathy of target organs such as brain, kidney, liver or the placenta. As a multisystem progressive disorder, preeclampsia involves maternal and feto-placental factors and is attributed to an abnormal vascular development in placentation in early pregnancy. This leads to a release of antiangiogenic factors into the maternal circulation that may alter maternal endothelial function and cause hypertension and other features of preeclampsia [2,3].

HELLP syndrome was first defined by Weinstein 1982 and is an acronym for haemolysis, elevated liver enzymes and low platelet count [3]. According to the ISSHP statement 2014, HELLP syndrome should be assumed in the presence of a low platelet count (≤100 G/l), a serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥2 times the upper limit of normality for local laboratory (usually ≥70 U/L), and as a consequence of haemolysis, lactic acid dehydrogenase (LDH) increase ≥600U/l [2,4]. These criteria are in accordance to the two major definitions used to diagnose HELLP syndrome, the Tennessee classification system, and the Mississippi triple class system [6–8]. However, in

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the Mississippi classification only class 1 and 2 are in accordance with the ISSHP statement while class 3 (platelet 100 to 150 G/L) is considered more as a transient form of HELLP syndrome [4]. Similarly, the Tennessee classification distinguish a complete form from an incomplete one. The latter one does not fulfil all three "classical" HELLP criteria.

1-2‰ of all pregnancies are complicated by HELLP syndrome. As stated by the ISSHP, HELLP syndrome should be considered as a severe form of preeclampsia and not as an isolated disorder [4,5]. However, isolated forms of HELLP syndrome without classical features of preeclampsia such as hypertension and proteinuria appear in approximately 16% of cases [6]. Moreover, HELLP syndrome may first occur only after delivery. Indeed, 30% are diagnosed in the postpartum period, usually within the first 48 h after delivery [7]. Of note, 80% of patients developing postpartum HELLP syndrome show signs of preeclampsia before delivery.

Nevertheless, the diagnosis of HELLP syndrome may not always be easy. Beside infectious complications or maternal medical diseases, other less common, but serious conditions such as immunologic thrombocytopenia (ITP), acute fatty liver of pregnancy (AFLP) or thrombotic microangiopathy (TMA) appearing as thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) may mimic HELLP [8].

Angiogenic profiling using the ratio between soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) has been shown to be helpful in differentiating pregnant women with clinical or laboratory signs for impending preeclampsia. However, little is known about the angiogenic profile of pregnancies complicated by HELLP syndrome as an isolated finding or in combination with preeclampsia. As HELLP syndrome is considered to be part of the preeclamptic syndrome spectrum, we would expect elevated sFlt-1/PlGF ratios in all cases of HELLP syndrome. The aim of this study was to examine the relationship of angiogenic profiles in cases of HELLP syndrome with and without apparent preeclampsia.

Materials and methods

This is a single centre prospectively collected and retrospectively analyzed observational study. From January 2011 to March 2018, all pregnant women admitted with preeclampsia as well as HELLP syndrome were included in our study. Blood analysis for angiogenic profiling was performed at admission. Diagnosis and treatment were not influenced by the angiogenic results. In cases where pregnancy prolongation was possible, angiogenic profiling was repeated at discretion of the clinical situation. For our analysis, we used the last angiogenic profile before delivery. Clinical and obstetrical data were retrieved from our electronic medical database.

Gestational age was based on to the last menstrual period usually confirmed or corrected by first trimester ultrasound scan. Preeclampsia was defined, according to the revised ISSHP (International Society for the Study of Hypertension in Pregnancy) statement 2014, as chronic or de novo hypertension after 20 weeks of gestation (blood pressure $\geq$140/90 mmHg in at least two measurements at least 15 min apart, preferably after overnight rest in hospital or in a day assessment unit) associated with significant proteinuria ($\geq$300 mg/24 h; spot urine protein/creatinine $\geq$30mmol/ml), or placental dysfunction with fetal growth restriction (FGR), or maternal organ dysfunction such as renal insufficiency, liver involvement, neurological or hematological complications [2,4]. FGR was defined as sonographic abdominal circumference <5th percentile or estimated fetal weight <10th percentile for gestational age with altered fetal and/or maternal hemodynamic or an abnormal growth trajectory over time [9]. Furthermore, small for gestational age (SGA) was defined as birth weight <10th percentile for gestational age.

Only women with platelet counts $\leq$ 100 G/l, liver enzymes $\geq$70U/L and LDH $\geq$ 600U/L were considered for the diagnosis of HELLP syndrome [4]. The enrolled women were subsequently substratified in three groups: Group 1 comprises pregnancies affected by isolated preeclampsia (PE), whereas group 2 consists of cases with preeclampsia associated with HELLP syndrome (PE/HELLP); finally, women with showing features of HELLP syndrome but without hypertension and proteinuria, later referred to as "isolated HELLP" were assigned to group 3 (HELLP). Exclusion criteria were multiple gestation and cases with fetal chromosomal and/or structural malformations. Cases with preeclampsia and low platelet count but without the full laboratory spectrum of HELLP syndrome so-called "partial or incomplete HELLP syndrome" were assigned to group 1 (preeclampsia) [4]. Women admitted with preeclampsia and developing HELLP syndrome later on, including postpartum, were assigned to group 2 (PE/HELLP).

Maternal serum sFlt-1 and PlGF concentrations were measured using electro-chemiluminescence immunoassays (ELECSYS; Roche Diagnostics GmbH, Mannheim, Germany) on cobas e 601 analyzer (Hitachi High Technology Co, Tokyo, Japan). To overcome gestational age dependent angiogenic behaviour, cases (group 3) were matched 1:2 with patients from group 1 as well as from group 2 and a subanalysis was performed. Matching criterion was gestational age ($\pm$ 1 week) at the time of blood analysis. If more than two patients fulfilled the matching criteria, patients who delivered the nearest to the index pregnancy were chosen.

Statistical analyses were performed with GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Continuous variables were analyzed using parametric and nonparametric tests, and ANOVA for multiple comparisons. Proportions were analyzed using Chi^2-test or Fischer's exact test where appropriate. A p-value of <0.05 was considered significant. The study was approved by the institutional ethics committee of the canton of Bern (Kantonale Ethikkommission Bern, Ref.-Nr.: 365/15).

Results

During the study period, 11146 women delivered in our clinic of whom 244 (2.2%) were diagnosed with preeclampsia and / or HELLP syndrome. 237 (97.1%) women fulfilled criteria for preeclampsia of whom 42 (17.2%) were associated with complete HELLP syndrome according to the Tennessee classification criteria. Only seven (2.9%) cases fulfilled the criteria of isolated HELLP syndrome, three belonging to Mississippi class 1 (platelet <50 G/L), and four to class 2 (platelet 50–100 G/L). In 152 (62.3%) cases preeclampsia developed <37 weeks, and in 117 (48.0%) cases <34 weeks of gestation. The clinical characteristics of the study population are depicted in Table 1. No significant clinical differences were found between the three groups. In the isolated HELLP group, one pregnancy had to be terminated due to severe threat of maternal health.

The angiogenic ratios were significantly different between the groups. Cases with isolated HELLP syndrome (group 3) showed the lowest median [range] angiogenic ratio compared to group 1, and group 2, respectively. This difference remained significant after correcting for gestational age by comparing age matched groups in a subanalysis. (median [range] sFlt-1/PlGF group 1 vs. group 2 vs. group 3: 108 [5-662] vs. 287 [51-948] vs. 49 [3-405]; p = 0.02).

Looking at the single angiogenic parameters sFlt-1 and PlGF, sFlt-1 was found to be the highest in group 2 and lowest in group 3, but not significantly different (median [range] sFlt-1 group 1 vs. group 2 vs. group 3: 9791 pg/ml [1122-42222] vs. 11578 pg/ml [1348-30781] vs. 7244 pg/ml [1648-25519]; p = 0.10). On the contrary, PlGF differed significantly between the groups with highest values in group 1 (median [range] PlGF in group 1 vs. group

Table 1
Clinical characteristics of the study population.

Characteristics	Group 1 Preeclampsia (n = 195)	Group 2 Preeclampsia/HELLP (n = 42)	Group 3 Isolated HELLP (n = 7)	Significance
Maternal age, years	31 (17–51)	30.5 (20–40)	33 (25–36)	p = 0.77
BMI, kg/m^2	26 (16.1–51)	24 (18–43)	24.5 (19–36)	p = 0.12
GA at delivery, weeks	33 (22–41)	32.9 (20.6–39.3)	35.9 (21.3–39.7)	p = 0.70
CS rate (n, %)	161 (85.2)	38 (90.4)	6 (85.7)	p = 0.66
Birthweight, grams	1620 (175–3955)	1523 (205–3240)	2180 (188–3200)	p = 0.60
Birthweight percentile	15.3 (0.8–99)	15 (1–75)	16.5 (3–73)	p = 0.48
SGA/FGR	76 (39.0)/70 (35.9)	17 (40.4)/17 (40.4)	3 (42.8)/3 (42.8)	p = 0.96/0.81
Umbilical cord pHart	7.31 (7.04–7.5)	7.33 (7.12–7.38)	7.36 (7.27–7.38)	p = 0.06
5'Apgar <7	32 (16.4)	8 (19)	1 (14.3)	p = 0.90
Perinatal mortality (n,%)	4 (2.1)	2 (4.2)	1 (14.3)	p = 0.12

Values are shown as median (range), or number and % were appropriate. GA, gestational age; CS, cesarean section; SGA, small for gestational age (Birthweight <10%ile); FGR, fetal growth restriction (sonographic abdominal circumference <5th percentile or estimated fetal weight <10th percentile with altered fetal and/or maternal hemodynamic or abnormal growth trajectory over time.

2 vs. group 3: 66 pg/ml [10-716] vs. 36 pg/ml [9-232] vs. 56 pg/ml [15-644]; p = 0.03). Of interest, comparing liver function tests between HELLP cases associated with preeclampsia (group 2) to those with isolated HELLP syndrome (group 3) no significant difference was found in aminotransferases. LDH as a marker for haemolysis was significantly higher in cases of HELLP associated with preeclampsia (group 2) than in isolated HELLP syndrome (group 3). (Table 2) Of note, no significant difference was found analyzing the angiogenic values subdividing the population of group 3 (isolated HELLP) according to the Mississippi classification system. No correlation was found between the platelet count and sFlt-1, or PlGF, or its ratio in both, group 2 and 3, respectively. However, significant correlations were found between PlGF and AST (r = −0.37; p = 0.01) as well as ALT (r = −0.39; p = 0.01) in group 2 while in group 3 (isolated HELLP syndrome) only AST showed a correlation with PlGF (r = −0.79; p = 0.02).

Comment

Pregnancies complicated by HELLP syndrome without apparent preeclampsia are characterized by a significantly different angiogenic profile than those with isolated preeclampsia or preeclampsia associated with HELLP syndrome. Indeed, cases with isolated HELLP syndrome show lower median sFlt-1/PlGF ratios due to lower median sFlt-1 values.

One major strength of our study is the strict use of inclusion criteria and in particular the definition of HELLP syndrome. Only patients matching all Mississippi or Tennessee criteria were included in our study. Moreover, we selected only patients with a platelet count of less than 100 G/l. In addition, this study was performed in a single centre, which guarantees more uniform diagnosis and treatment strategies. A weakness of our study however is the relatively small number in sample sizes. Only seven patients were diagnosed with isolated HELLP syndrome.

Table 2
Laboratory findings of cases with isolated HELLP syndrome and those associated with preeclampsia (group 2 and 3).

Laboratory characteristics	PE/HELLP (n = 42)	Isolated HELLP (n = 7)	Significance
Tc (G/L) (ref. 150-450)	60 (5–100)	56 (21–79)	p = 0.45
AST (U/l) (ref. < 35)	293 (37–3917)	185 (54–552)	p = 0.14
ALT (U/l) (ref. < 35)	251 (56–2953)	180 (54–262)	p = 0.18
LDH (U/l) (ref. < 480)	1030 (625–16892)	669 (642–1342)	p = 0.01*
sFlt-1/PlGF ratio, IQR	282 (7–948), 335	49 (3–405), 110	p = 0.01*

Values are shown as median (range). Tc, thrombocytes; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic acid dehydrogenase; sFlt-1/PlGF, soluble fms-like tyrosine kinase-1/placental growth factor; IQR, inter quartile range.
Continuous variables were analyzed using nonparametric tests (Kruskal Wallis). Proportions were analyzed using Chi^2-test or Fischer's exact test.

Most studies do not differentiate between isolated HELLP syndrome and preeclampsia accompanied by HELLP syndrome, making a comparison difficult [10–12]. Similar to our findings, Muñoz-Hernández et al. found that women with preeclampsia complicated by HELLP syndrome showed the highest sFlt-1/PlGF ratios [10].

Verlohren et al. reported on angiogenic profiles of patients diagnosed with preeclampsia. Their study contains a group of pregnancies with isolated HELLP syndrome. In contrast to our analysis, patients with isolated HELLP had the highest sFlt-1/PLGF values compared to isolated preeclampsia and preeclampsia associated with HELLP syndrome. They report briefly about the definition used to define HELLP syndrome and leave space for interpretation. This point may explain why the prevalence of isolated HELLP syndrome in the mentioned study was higher than in our study (n = 15 cases, 6% of the population) [11,12]. Moreover, isolated HELLP syndrome cases were not matched according to gestational age to cases with preeclampsia or preeclampsia complicated by HELLP syndrome as we have chosen to do in our study. Therefore, this apparent discrepancy between our results and those described in the study of Verlohren et al. has to be interpreted with caution.

The PROGNOSIS study as well as the post hoc analysis by Zeisler et al. reported a sFlt-1/PlGF cut-off of <38 to rule out the development of preeclampsia within the next week in cases with signs for impending preeclampsia with a negative predictive value of 99.3% [13,14]. Accordingly, higher cut-off values were associated with increased risks to develop preeclampsia. However, the positive predictive value remains elusive. Verlohren et al. proposed different cutoff values of the sFlt-1/PlGF ratio to increase the diagnostic accuracy. Indeed, they found that an sFlt-1/PlGF ratio ≥85 has as high specificity in cases ≤34 weeks of gestation while in later gestational age (>34 weeks) a ratio ≥110 yield the best specificity [12]. It has been postulated that this different gestational age dependent angiogenic performance may reflect the pathophysiological and clinical difference postulated for early and late preeclampsia. Similarly, our results may indicate, that "isolated HELLP syndrome" may be a form of preeclampsia that first presents itself through changes in liver metabolism. This form is also associated with angiogenic imbalances but to a lower extend than in preeclampsia or preeclampsia with HELLP syndrome.

HELLP syndrome associated with preeclampsia seems more to mark a progressive deterioration of the preeclamptic syndrome. Indeed, the only difference between preeclampsia with and without HELLP syndrome in our cohort was an increased sFlt-1/PlGF ratio due to both, increased sFlt-1 and reduced PlGF serum

levels in these patients marking an aggravation of the already manifest anti-angiogenic status in preeclampsia. Thus, the placental release of sFlt-1 is increased upon these stress factors [15] while PlGF serum levels are decreased [16].

Of particular interest is the finding, that the platelet count - which is central for diagnosis of HELLP syndrome and even for defining its severity - does not correlate with the angiogenic information while aminotransferases, and in particular AST, shows a negative associated behavior with PlGF. However, the small number of cases limits further interpretation of this finding.

HELLP syndrome shares some of the pathophysiological characteristics of other thrombotic microangiopathies (TMA) such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) which include endothelial injury, platelet aggregation, microthrombi, thrombocytopenia, and anemia [17]. The lower angiogenic ratio we found in our cohort of isolated HELLP is intriguing and may suggests a possible similarity with TMA This may be of importance in differentiating HELLP syndrome from TMAs. This point has been investigated recently by Gupta et al. and a new algorithm has been proposed for that purposes [18]. While ADAMTS13 is helpful in diagnosing TTP but not readily available, the distinction of HELLP syndrome from atypical hemolytic uremic syndrome (aHUS) can be even more challenging. However, little is known about angiogenic profiles in TMAs other than isolated HELLP syndrome or association with preeclampsia. Further studies are required to investigate angiogenic behavior of TTP and aHUS.

In conclusion, our data demonstrate that pregnant women with isolated HELLP syndrome have a distinct angiogenic profile pattern, which is different from that observed in patients with preeclampsia. This discrepancy underscores that so called isolated HELLP syndrome may be a form of preeclampsia first becoming apparent through changes in liver metabolism. Furthermore, angiogenic characterization may be of importance in distinguishing isolated HELLP syndrome from other thrombotic microangiopathies.

Disclosure statement

The authors report no conflict of interest

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