Diplomarbeit

# Exertion of a combined therapeutical concept: Influence on pregnancy outcome and clinical parameters in HELLP patients


submitted by

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attaining the academic degree

**Doktor der gesamten Heilkunde**

(Dr. med. univ.)

at the

**Medical University Graz**

conducted at the

**University Clinic for Gynecology and Obstetrics Graz**

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Graz, 28st January 2015

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Graz, den 28. Januar 2015 Christiane Sylvia Barthel eh.

# Danksagungen

Diese Arbeit wäre nicht möglich gewesen, ohne die entspannte und kompetente Betreuung durch Dr. Ioana Lakovschek. Danke für deine Geduld, deine viele Zeit und deine Unermüdlichkeit. Es war eine Freude, mit dir zu arbeiten. Herrn OA Dr.med. Csapo danke ich für die guten Verbesserungsvorschläge und Hilfestellungen, vor allem am Schluss. Dank gilt auch Prof. Mila Cervar-Zivkovic für die Betreuung und Bewertung meiner Arbeit.

Die statistische Analyse, die für diese Arbeit notwendig war, habe ich von Frau Sereina Herzog gelernt. Ich möchte ihr gerne danken, für ihren Willen meine Daten zu verstehen und mir die Wirrungen von SPSS näher zu bringen. Ohne sie wäre das nichts geworden.

Dank gilt hier auch meinen Korrektoren Karin Thurnher, Michael Lukesch und Tobias Barthel. Vielen Dank für eure Zeit, obwohl ihr selbst so viel um die Ohren habt.

Die Schönheit meiner Grafiken ist der Hand meiner guten Freundin Bernadette Sattler-Remling entsprungen. Danke dafür, aber auch fürs Unterstützen, Gut-zu-reden und mich Verstehen, während diese Arbeit am Entstehen war. Du bist einfach super.

Danke auch an meine Familie: Mum, Doro, die Tanten und die Barthels für euren Glauben daran, dass ich das kann. Tobias, du bist mein Held gewesen in den letzten eineinhalb Jahren. Danke für deine Geduld, deine Hilfe, deine Liebe. Ich hätte es nicht ohne dich geschafft.

# Contents

# Abbreviations

| | |
|---|---|
| ACTH | Adrenocorticotropic hormone |
| ADP | Adenosine diphosphate |
| AFLP | Acute fatty liver of pregnancy |
| ALT | Alanine transaminase |
| AP-1 | Activator protein 1 |
| APLS | Antiphospholipid syndrome |
| AST | Aspartate transaminase |
| BMI | Body mass index |
| BP | Blood pressure |
| CNS | Central nervous system |
| CPK | Creatine phosphokinase |
| CRH | Corticotropin-releasing hormone |
| CRP | C-reactive protein |
| CT | Computed tomography |
| DIC | Disseminated intravascular coagulation |
| DM | Diabetes mellitus |
| EL | Elevated liver enzymes |
| ELLP | Elevated liver enzymes, low platelets |
| FGR | Fetal growth restriction |
| FVL | Factor V Leiden |
| GA | Gestational age |

| | |
|---|---|
| GR | Glucocorticoid receptors |
| HELLP | The syndrome of hemolysis, elevated liver enzymes and low plateles |
| HUS | Hemolytic uremic syndrome |
| ICU | Intensive Care Unit |
| ID | Identity number |
| IL | Interleukin |
| ITP | Idiopathic thrombocytopenic purpura |
| IUGR | Intrauterine growth restriction |
| iv. | Intravenous |
| IVF | In vitro fertilisation |
| LDH | Lactate dehydrogenase |
| LP | Low platelets |
| LR | Likelihood |
| MTHFR | Methylenetetrahydrofolate reductase |
| NF | Transcription factor |
| NO | Nitric oxide |
| PE | Preclampsia |
| PIGF | Placental growth factor |
| PP 13 | Placental protein 13 |
| RR | Riva-Rocci (Blood pressure) |
| RUQP | RUQP |
| SIRS | Systemic inflammatory response syndrome |
| SLE | Systemic lupus erythematosus |
| SNP | Singe-nucleotide polymorphism |
| STBM | Syncytiotrophoblast particles |
| TC | Thrombocytes |

| | |
|---|---|
| TNF | Tumor necrosis factor |
| VEGF | Vascular endothelial growth factor |
| VWF | Von Willebrand factor |
| WHO | World Health Organization |

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# Zusammenfassung

**Einleitung** Das HELLP Syndrom (Hämolyse, erhöhte Leberwerte und Thrombozytopenie) gehört zu den schweren Schwangerschaftskomplikationen und ist mit hoher maternaler und kindlicher Morbidität verbunden. Es tritt in 0,2-0,8% der Schwangerschaften auf und kann derzeit nur durch die Entbindung kausal behandelt werden. Viele Studien zeigen einen positiven Effekt von hochdosierten Glukokortikoiden auf den Krankheitsverlauf und die Komplikationsrate in HELLP-Patientinnen. Zusätzlich dazu, zeigt sich die intravenöse Gabe von Magnesiumsulfat als wichtiger Faktor bei der Prophylaxe von Eklampsie. Glukokortikoidtherapie und Magnesiumadministration sind Teil unseres multimodalen Therapiekonzeptes für HELLP Syndrom, das wir mit dieser Studie evaluiert haben.

**Methoden** Zwischen 2005 und 2013 wurden von HELLP-Patientinnen der Universitätsklinik für Gynäkologie und Geburtshilfe Graz in die Studie eingeschlossen. In einer retrospektiven Studie wurde der Effekt von hochdosiertem Prednisolon auf den Krankheitsverlauf, Schwangerschaft das Kind untersucht. Die Daten wurden in absoluten Zahlen und Prozenten angegeben. Die statistische Analyse wurde mit SPSS durchgeführt wobei der Chi-Quadrat-Test, Fisher's-Test, T-Test und ANOVA-Calculation verwendet wurden. Ein zweiseitiger P-Wert unter 0.05 wurde als significant angenommen.

**Ergebnisse** Wir haben 80 Frauen mit HELLP Syndrom in diesem Zeitraum gefunden (n=80), welche in drei Gruppen einzuteilen waren:

PRED hochdosiertes Prednisolon wurde ein bis dreimal verabreicht ($n = 50, 62.5\%$)

MIX bekamen mehr als drei Dosen, auch von unterschiedlichen Cortisonderivaten($n = 12, 15\%$)

NO, keine Glukokortikoidtherapie (n=18, 22.5%)

Tendenziell hatten Frauen in der PRED-Gruppe einen kürzeren Aufenthalt im Krankenhaus und eine kürzere Zeit auf der Intensivstation. Zusätzlich waren tendenziell weniger

Kaiserschnitte und mehr vollständig druchgeführte Lungenreifebehandlungen in unserer Hauptgruppe zu vermerken.
Unabhängig von Cortison-Gruppen wurden die Patientinnen in Magnesium- und Kein-Magnesium-Gruppen eingeteilt:

Mg Patientinnen mit intravenöser Magnesiumsulfat-Therapie
NoMg Patientinnen ohne intravenöser Magnesiumsulfat-Therapie

In der Magnesium-Gruppe war die Eklampsierate tendenziell niedriger als in der Gruppe, die kein Magnesium erhalten hat.

**Zusammenfassung** Unsere Studie konnte die bisherige Datenlage bestätigen, dass sich Magnesiumsulfat positiv auf die Eklampsierate auswirkt. Daraus folgernd stellt Magnesiumsulfat einen wichtigen Therapiansatz beim HELLP Syndrom dar. Auch wenn nicht statistisch signifikant, scheint eine hohchdosierte Prednisolontherapie sich positiv auf die Komplikationsrate und Krankheitsverlauf bei HELLP-Syndrom-Patentinnen auszuwirken. Wobei das konservative Management im multimodalen Kozept an einem tertiären Zentrum erfolgen soll. Weitere größere prospektive Studien müssen folgen, um die hochdosierte Kortikosteroidtherapie als Teil dieses Therapie-Konzeptes zu evaluieren.

# Abstract

**Introduction** The syndrome of hemolysis, elevated liver enzymes and low platelets is a severe complication of pregnancy. It is associated with a higher risk of complications in mother and child, and effective ways for conservative management are yet to be developed. Glucocorticoids have been claimed to be beneficial in HELLP patients for pregnancy outcome and disease course. Also, administration of magnesium sulfate showed a positive effect on prophylaxis of eclamptic seizures. Glucocorticoids and magnesium application are therefore part of our combined therapeutical concept for HELLP syndrome, and their effects have been evaluated in this study.

**Methods** From the years 2005 until 2013 the effect of high dose prednisolone on maternal and neonatal outcome in HELLP syndrome was analyzed in a retrospective study. Data was presented in absolute numbers and percentages. Statistical analysis was performed using Chi square, Fisher's exact test, independent T test and ANOVA calculation. A two-sided p-value of less than 0.05 was considered as significant.

**Results** Pregnancies complicated by HELLP syndrome (n=80) were divided in 3 groups:

PRED receiving high-dose prednisolone administered one to three times ($n = 50, 62.5\%$)
MIX received more than three doses and different types of corticosteroids ($n = 12, 15\%$)
NO, receiving no corticosteroids (n=18, 22.5%)

There is a tendency to shorter intensive care and hospitalization time, decreased incidence of cesarean section, and increased ratio of completion of lung maturation in PRED group. Independently from these groups, the cohort was distributed into Mg and NoMg groups.

Mg group Patients with intravenous magnesiumsulfate infusion
NoMg group Patients without intravenous magnesiumsulfate infusion

The rate of occured eclampsia and neurological deficiency was determined within these groups. We found a tendency of decreased occurance of eclampsia and can assert that magnesium sulfate infusion is beneficial for HELLP patients.

**Conclusion** We confirmed that magnesium sulfate has a positive effect on eclampsia rates. Therefore it is an important asset in the therapeutical management of HELLP syndrome. Although no significant results were found, high-dose prednesolone application seems to be beneficial for complication rate and course of disease in HELLP patients. Conservative management with this combined therapeutical concept should be performed at a maximum care facility. Bigger prospective studies must follow to further evaluate high-dose corticosteroid administration in HELLP patients.

# 1 Introduction

## 1.1 Purpose of study

The syndrome of hemolysis, elevated liver enzymes and low plateles (HELLP syndrome) is a serious complication of pregnancy, often referred to as a form of severe preeclampsia[1]. It poses a great threat to mother and child, because morbidity and mortality rates in affected wome are high. On the one hand, this condition is related to a higher risk of several complications in the mother and the child is most affected by HELLP when onset of the disease is very early in pregnancy, considering complications that are associated with premature birth. Even though neonatal care has improved exponentially in the last decade, preterm delivery is often followed by neonatal morbidity and a high mortality rate[55][41]. 20 years ago, researchers introduced the application of glucocordicoids to prolong pregnancy in order to improve the maternal and neonatal outcome. Initially given for lung maturation at impending delivery before the $34^{th}$ gestational week, they saw that the status of mothers has stabilized as well. Therefore corticosteroids were proclaimed to be beneficial for maternal outcome. Expectant management is only possible though, when the status of fetus and mother is stable and without further complications. Delivery that is induced in an acute exacerbation of HELLP could worsen the situation for mother and child. It is crucial in management of HELLP to balance between the risk of preterm delivery for the child, and acute and possibly permanent damage for the mother[7][41][55].

Based on previous literatur regarding corticosteroids in management of HELLP syndrome, corticosteroid use was introduced to our department in 2006 [62][65]. The therapeutic concept includes high-dose prednisolone administered one to three times starting at the time of diagnosis, similar to the treatment of anaphylaxis. One goal is to reach a high serum concentration of prednisolone, in order to achieve the best possible effect. Prednisolone (0.5 to 1 g) one to three times at the most are administered as bolus transfusion, therefore avoiding the problem of adrenal suppression. This side-effect can be

caused by long-term corticosteroid exposure. Prednisolone crosses the placental barrier in small concentrations only, minimizing the negative effects of corticosteroid therapy for the fetus [62][65].

In order to evaluate our therapeutic management, we conducted a retrospective study investigating all cases of HELLP syndrome between January 1st 2005 and December 31st 2012. The study analyzed the impact of a multilayered therapeutic concept that consists of high-dosed cortisone treatment, hypertension control and magnesium sulfate administration. This concept focuses on stabilizing HELLP patients by targeting hypertensive and neurological dangers, as well as inflammation that accompany patients with HELLP. We compared disease course, outcome of patients and immediate newborn data from pregnancies with HELLP syndrome, with or without high-dose prednisolone therapy. Apart from that, this study also intents to show the beneficial effect of intravenous magnesiumsulfate administration in these patients. Researchers found that magnesium sulfate transfusion decreases the incidence of eclamptic seizures and neurological symptoms [16][68].

## 1.2 Definition of HELLP syndrome

HELLP syndrome was named for the first time and defined as a form of severe preeclampsia by in 1982 Weinstein et al. He stated that HELLP is the syndrome of hemolysis or microangiopathic hemolytical anemia (H), elevated liver enzymes (EL) and low platelets (LP) [69][55]. Preeclampsia is proclaimed to be newly obtained hypertension (≥140mmHg and ≤90) restricted to the time of pregnancy after the 20th gestational week (GA) in combination with proteinuria (≥ 300 mg/day). Severe PE is defined as pregnancy induced hypertension and proteinuria in combination with one of the following criterias [15]:

- Hypertension ≥ 160/110 mmHg
- Signs of renal failure (Creatinin ≥ 0.9 mg/dl) Oliguria (≤ 500 ml in 24 hours)
- Elevated liver enzymes
- Pulmonary endema
- Hemolysis or thrombocytopenia
- Neurological symptoms

– Fetal growth restriction

PE is ten times more common than HELLP syndrome [1]. In this thesis hemolysis will be mainly defined by elevated LDH levels ($\geq 243\ IU\ l$) in maternal blood. However, it is also possible to use Haptoglobine, indirect bilirubine and abnormal peripheral smear (schistocytes, burrcells, echinocytes) as parameter for hemolysis [55]. Most researchers use elevated aspartate transaminase (AST) or alanine transaminase (ALT) as a reference for liver enzymes. Put together, high ALT as well as AST levels of more than 70 IU l, platelet counts under 100000 $/\mu\, l$ and high LDH were the definition of Sibai et al. to secure diagnosis of HELLP. We used these parameters to determine HELLP Syndrome in our patients as well [55]. Below, the syndrome itself will be discussed further incorporating recent scientific findings.

## 1.3 Pathogenesis

Researchers around the world have not yet found what actually causes HELLP syndrome and how it develops. This syndrome complicates $0.2 - 0.8\%$ of pregnancies showing a pathogenesis very similar to Preeclampsia and other placental dysfunctions [1]. Like PE it seems to be caused by ischemic placental aberration and dysfunction [47][41]. In table 1.1 the pathological development of PE is illustrated. It shows the influence of placental and maternal factors on the endothelial system of the mother in PE. As mentioned earlier, this pathological pathway is to also broadly applicable to HELLP syndrome. The liver-emphasized inflammatory process and activation of coagulation associated with HELLP is, however, to a greater degree more distinctive and severe in HELLP than in PE. It seems a disordered immunological process is involved in the pathogenesis of this excessive inflammatory response in HELLP. This is probably based on a preexisting genetic susceptibility (see also subsection 1.3.3) [9][41]. Placental emissions probably cause inflammatory cytokines to be released into maternal bloodstream and start an inflammatory response in the mother [41][1]. Several genetic and pathogenetical aspects are illustrated and discussed in more detail in the following subsections.

### 1.3.1 Genetics

No underlying genetic cause has been found to be the only aspect increasing the risk for HELLP. Though some findings suggest that certain gene mutations, each for itself present-

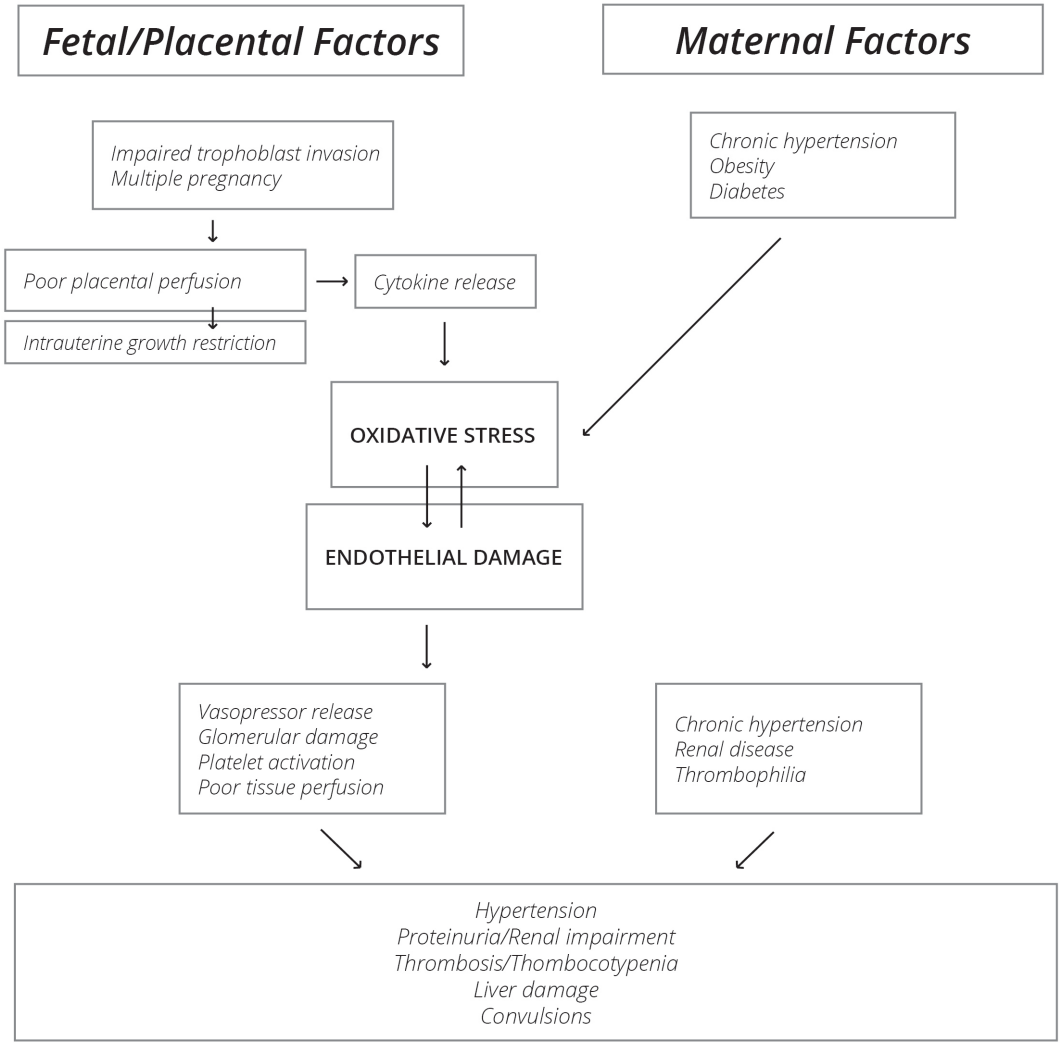


Figure 1.1: Assumed Pathophysiology of Preeclampsia [11]

ing a mild risk could be understood as a probable etiology if coexisting simultaneously, and combined with environmental and maternal factors. In table 1.1 these suspected gene variants are listed and in table 1.3.2 their implications on the development of HELLP are further explained.

Variants in Fas[1], the vascular endothelial growth factor (VEGF) gene, and the coagulation factor V Leiden (FVL) mutation are considered to be responsible for increasing the risk of HELLP compared to women without these mutations [1][47][60][45]. Mutations in complement inhibitor genes result in dysfunction of complement activation and therefore increase the risk of these women to develop preeclampsia [52]. Additionally, variations

[1]Genes for TNF receptors

| Gene variant | Compared to | Effect |
|---|---|---|
| Glucocorticoid receptor gene, | Healthy pregnant | Altered immune sensitivity and |
| Bell SNP polymorphisms | Severe PE | glucocorticoid sensitivity |
| Toll-like receptor 4 gene, | Healthy pregnant | Uncontrolled or harmful |
| $D299G$,$T3991$ polymorphisms | PE | inflammation, ineffective immunity |
| VEGF gene | Healthy pregnant | Angiogenesis and vasculogenesis, |
| $C-460T$ | Healthy pregnant | arterial muscular relaxation |
| $G+405C$ polymorphisms | | |
| FAS ($TNFRSF6$) gene | Healthy pregnant | Immune regulation, apoptosis, |
| | | Liver disease |
| Factor V Leiden | Healthy pregnant | Thrombophilia |

Table 1.1: Genetic variants connected with an increased risk of HELLP [1][47][60][45]

in glucocorticoid receptor gene and the toll-like receptor gene seem to cause a higher risk for HELLP than they do for PE [1].

### 1.3.2 Placental Pathogenesis

In HELLP the patient's development and placentation of the placenta is disordered [41][1]. This is also called the placental stage of HELLP. The aberrant placental function results in systemic reactions, as it produces cytokines and other substances, and sheds them into the maternal blood stream [11][1]. Moreover, certain genetic variations also influence this response and therefore predispose women for HELLP (see subsection 1.3.1).

As mentioned in subsection 1.3.1, variations in Fas genes seem to be important for the pathogenesis of HELLP. Fas and its ligand FasL are transmembrane proteins that are assigned to the tumor necrosis factor superfamily. One of their tasks is to mediate the invasion of the fetal extravillous trophoblast into uterine wall blood vessels, as well as to uphold the maternal tolerance of fetal cells. Activated lymphocytes for example, are cells that produce Fas. The expression of FasL though, is confined to designated leukocytes and the human trophoblast. Normally, binding of these FasL-carrying trophoblasts with Fas-expressing maternal T lymphocytes during implantation results in the latter undergoing apoptosis. That makes a deep myometrial implantation possible without immune recognition. Gene variants in Fas result in dysfunction of the regulation of these processes, followed by an inadequate implantation. For this reason in particular maternal T cells

also express FasL and hence induce apoptosis in trophoblast cells. They are subsequently limited in their implantation capabilities [60].

Fas ligand CD95L is produced by the placenta and is important for the development of HELLP. Liver cells express CD95 in large numbers making them sensitive to the excessivly elevated CD95L, circling in the blood stream of HELLP patients. CD95 stimulation by CD95L provokes the production of $TNF\alpha$ in liver cells and causes their apoptosis and necrosis. CD95L is therefore responsible for the damage in hepatocytes as it is elevated in maternal blood of HELLP patients as opposed to healthy pregnant women [1][59].

### Biomarkers

In this subsubsection biomarkers are discussed, that are likely to be associated with PE and early onset HELLP syndrome. Some of these 'early Biomarkers' and their concentrations and functions during pregnancy are summarized in 1.2.

Researchers suspected a defect syncytiotrophoblast membrane is the reason why an increased amount of syncytiotrophoblast microparticles is found in maternal blood samples of HELLP patients [21]. This syncytiotrophoblast membrane has a deformed morphology at its brush border. Its foremost function lies in the separation of maternal and fetal blood. The contained placental protein (PP 13), for example, is responsible for the development of maternal/fetal interface and immune regulation during placentation. High PP 13 concentration in the last trimester in maternal blood suggests that the aponecrotic syncytiothrophoblast brush border sheds it into the maternal blood stream. Reduced PP 13 (compared with healthy pregnancies) in the first three months of a PE or HELLP pregnancy implies that the development of these syndromes sets in early in the first trimester. This difference in serum concentration in gestational weeks 8 to 14 suggests a dysfunctional process in placental genesis in PE and HELLP patients. It is therefore one of the few biomarker capable of predicting even late onset PE very early in pregnancy (7 to 8 week of gestation). Additionally, VEGF is an important angiogenetic factor and modulator of the proliferation of endothelial cells and induces vasculargenesis and regulates vascular permeability. Hypoxia is the main trigger for VEGF production in many cell types that produce it, in this case cytotrophoblasts. Nagy et al. found genetic polymorphisms, such as $C-460T$ and $G+405C$, which are associated with the risk of HELLP (see subsection 1.3.4) [47].

Anti-angiogenetic biomarkers are elevated in HELLP as well (see table 1.2), and are related to the induction of vascular endothelial dysfunction in the mother. This may cause

| Biomarker | GA | HELLP | PE | Function of marker |
|---|---|---|---|---|
| PP 13 | 8-14 | ↓ | ↓ | Development of fetal/ |
| placental protein 13 | 24-37 | ↑ | ↑ | maternal interface, |
| | | | | immune regulation |
| PIGF | 8-14 | ↓ | ↓ | Angiogenic, |
| Placental growth factor | Term | ↓↓ | ↓ | prevents hypertension |
| VEGF | 14-21 | n.e. | ↓ | Angiogenic, |
| vascular endothelial growth factor | 32 | ↑ | ↑↑ | prevents hypertension |
| sFlt1 | 10-17 | n.e. | ↑ | Inhibits VEGF and PIGF, |
| solutable fms-like tyrosine kinase | 25-40 | ↑↑ | ↑ | Anti-angiogenic |
| sEndoglin | 10-17 | n.e. | ↑ | Inhibits TNF-$\beta$ and |
| | Preterm | ↑↑ | ↑ | Vasodilatation, |
| | Term | ↑ | ↑ | Anti-angiogenic |

n.e. = not examined; ↑ ≙ higher than in pregnant controls ($p \leq 0,05$), ↑↑ ≙ higher than ↑ ($p \leq 0,05$)

Table 1.2: Biomarkers in maternal blood predicting early-onset HELLP or PE [1]

arterial hypertension and glomerular endotheliosis. The anti-angiogenic factors sendoglin and sFlt1 are linked to an elevated immune activation, like high levels of TNF$\alpha$ and interleukin-6. Solutable Flt1 is closely associated with the severety of preeclampsia and therefore could be used to prognosticate it during pregnancy [68]. Not like pp 13 though, it can predict PE only some weeks before clinical onset of symptoms, and can also be elevated in IUGR pregnancies [24]. An elevated sFlt1/PIGF ratio is claimed to be useful for predicting pathological pregnancies including PE and IUGR some weeks before clinical onset. Other than that, it could be benificial for differential diagnosis (see subsection 1.4.3) [53]. As shown in table 1.2 this ratio may also be increased in early onset HELLP patients. This is, however, still the subject of research.

The importance of these biomarkers lies in prediction of PE or HELLP syndrome as early as possible in pregnancy. This would make it possible to start medication and use preventive strategies [25] [1]. It is suggested to also take blood pressure, Doppler flow velocity waveforms of the uterine artery, and maternal history into account for diagnosis. Biomarkers which could HELLP/PE are object of intensive research at the moment. They may lead to more intense surveillance and to a better clinical outcome for patients [70][24].

### 1.3.3 Pathogenesis in the mother

#### The inflammatory response

In HELLP patients the inflammatory response is increased compared to the inflammatory response in PE patients [34]. In the process of pathogenesis complement, coagulation and the inflammatory system are activated supposedly by syncytiotrophoblast particles (STBM). While circulating in the maternal blood system, STBM and other placenta-derived substances react with the vascular endothelial and immune cells and basically cause an inflammatory reaction. A dysfunction of the complement may lead to development of thrombotic microangiopathy and result in endothelial dysfunction as well [41].

In women with HELLP levels of CRP, Interleukin 6 and TNF$-\alpha$, as well as the white cell blood counts are higher than in patients with PE. The later corresponds to the severity of the particular illness [1]. Additionally, Hulstein et al. showed that the activation of endothelial cells causes them to release active von Willebrand factor (VWF). VWF is responsible for increased intravascular platelet aggregation [23].

#### Thrombotic microangiopathy

As mentioned before thrombotic microangiopathy is found in HELLP patients. This might be caused by an interaction of high TNF$-\alpha$ levels, too much active VWF and other anti-angiogetic substances, like shown in table 1.2, in maternal blood. The elevation of active VWF results from decreased enzymatic metabolism into the inactive structure, as the responsible enzyme (ADAMT13) is reduced in HELLP patients [1]. High active VWF levels cause thrombotic microangiopathy, which leads to usage of platelets and could therefore explain the trombocytopenia typical for HELLP [23].

#### Hemolytic anemia

As mentioned before, vascular endothelium in the mother is damaged subsequently causing erythrocytes to be destroyed. This leads to microangiopathic hemolytic anemia and elevated LDH values [1]. Thus, the peripheral smear is pathological: schistocytes, burr cells and echinocytes are found [55]. These damaged erythrocytes in the bloodstream may increase the risk of disseminated intravascular coagulation (DIC) through possibly activating coagulation. Another pathway for DIC is the interaction of fetal microparticles with tissue factor, which is one of the most important activators of coagulation. Hence,

coagulation processes are actuated. The uptake rate of coagulation inhibitors is boosted and DIC becomes more probable [1].

#### Liver and kidney dysfunctions

The above-quoted FasL(CD95) causes definitive death of hepatocytes, as it triggers a cytotoxic reaction [59]. Autopsies have indicated that fibrin and leukostasis were deposited in HELLP liver sinusoids. This is caused by thrombotic microangiopathy, as explained in1.3.3. Furthermore, this microangiopathy reduces portal blood flow in women with HELLP; something that doesn't occur in patients with severe PE without HELLP [1]. Usually, as in PE, HELLP patients have mild renal dysfunction probably induced by glomerular endotheliosis typical for PE. The above-mentioned thrombotic microangiopathy is most likely the origin for severe renal dysfunction in patients with HELLP [1].

To sum up, these findings suggest that HELLP syndrome forms early in first trimester in the stage of placentation. On the one hand, placenta-derived proteins lead to oxidative stress and endothelial dysfunction in the mother. This may cause hypertension, thrombocytopenia and hemolysis [11][1]. On the other hand, they damage hepatic cells, causing an inflammation impaired by disordered immunologic reaction in the mother. Liver cells subsequently die in that process. Furthermore there are several congruencies between HELLP and systemic inflammatory response sydrome (SIRS). For example, increased proinflammatory mediators, such as cytokines, are also elevated in early stages of SIRS [8][41].

### 1.3.4 Risk factors

Some risk factors have been found for PE, but have not been significant for HELLP. For instance a high body mass index (BMI), diabetes and chronic hypertension are considered to be a risk factor for PE. However, it is not known whether they are also risk factors for HELLP [11]. In contrast to PE, nulliparity is not a risk factor for HELLP syndrome [4]. Risk factors for PE are summarized in table 1.3.

Sisters and daughters of women who suffered from HELLP carry a higher risk of developing HELLP [1]. In genetic studies some genes were found to increase the risk of HELLP. The Fas gene mentioned in subsection 1.3.2, namely the homozygous TNFRSF6 polymorphism $TNFRSF6*G$, was found to elevate the risk for early onset preeclampsia and intrauterine growth restriction. Furthermore, this study showed that patients with heterozygous and homozygous $TNFRSF6*G$ allele were more likely to get HELLP than

| Risk factor | Risk ratio (RR) |
|---|---|
| Anti-phospholipid syndrome | $\approx 9$ |
| Previous PE | $\approx 7$ |
| BMI $\geq 30$ | $\approx$ 3-5 |
| Diabetes mellitus | $\approx 3.5$ |
| Mother or sister having had HELLP | $\approx 3$ |
| Kidney disease | $\approx 3$ |
| Nulliparity | $\approx$ 2.5-3 |
| Age over 40 years | $\approx 2$ |
| Chronical hypertension | RR $\uparrow$ |
| Autoimmune diseases | $\approx$ 7-9.7 |
| Ethnic backround (afro-american) | $\approx 2$ |
| Bilateral notching after 24 gestational weeks | LR $\approx$ 3.4-6.5 |
| Multiple pregnancy | $\approx 3$ |
| IVF | RR $\uparrow$ |
| Pregnancy related diabetes, Hydrops fetalis, trinominal fetus, hydatid mole | |

LR = Likelihood ratio

Table 1.3: Risk factors for PE [14][10][5][15]

those with homozygous wild type Fas gene ($TNFRSF6*A$). However, there was no connection found between the genotype and the severity of HELLP [60]. As shown in subsection 1.3.2 the carriers of genotypes VEGF $-460TT$ and $+405CC$ have an increased risk of acquiring HELLP [47]. Anti-phospholipid syndrome (APLS),however, could be linked to early onset HELLP syndrome [1]. Additionally patients with APLS and HELLP could develop multiorgan failure, which is called catastrophic APLS [32].

## 1.4 Diagnosis

HELLP syndrome typically occurs suddenly in the $27^{th}$ to $37^{th}$ gestational week or instantly postpartum ($15-25\%$ of cases). Although in 20 - 30% it manifests already before the $28^{th}$ week of gestation, where it is defined as early onset HELLP syndrome [41][1]. For patients with preeclampsia, incidence of HELLP lies between $2-12\%$ [7]. In $70-80\%$ of

patients with HELLP additional hypertension and proteinuria has been diagnosed, namely the 2 main clinical signs of preeclampsia [1][41].

### 1.4.1 Laboratory presentation

| **Class** | Mississippi classification | Tennessee classification |
|---|---|---|
| 1 | Platelet count $\leq 50000/\mu l$<br>AST or ALT $\geq 70 IU/l$<br>LDH $\geq 600 IU/l$ | Platelet count $\leq 100000/\mu l$<br>AST or ALT $\geq 70 IU/l$<br>LDH $\geq 600 IU/l$ |
| 2 | Platelet count $\geq 50000/\mu l$<br>and $\leq 100000/\mu l$<br>AST or ALT $\geq 70 IU/l$<br>LDH $\geq 600 IU/l$ | |
| 3 | Platelet count $\geq 100000/\mu l$<br>$\leq 150000/\mu l$<br>AST or ALT $\geq 40 IU/l$<br>LDH $\geq 600 IU/l$ | N/A |
| Partial HELLP/<br>Incomplete HELLP | N/A | Severe preeclampsia and<br>one of the following symptoms:<br>ELLP, EL, LP |

Table 1.4: Classification of HELLP syndrome [41]

Table 1.4 shows the two main classifications. The Mississippi Classification describes 3 classes, whereas the allocation depends mainly on platelet count. Class 1 is considered the most severe specification of HELLP with platelet counts under 50000 $/\mu l$, ALT or AST values greater than 70 $UI/l$ and LDH values greater than 600 $IU/l$ [41]. Class 2 subsums all cases with a platelet count between 100.000 to 150.000 $/\mu l$ and class 3 with thrombocytes over 150.000 $/\mu l$ with the same alterations in AST/ALT and LDH levels. The Tennessee classification only distiguishes between two classes: Class 1, which corresponds to class 2 of the Mississippi classification and partial HELLP. The latter is defined as a syndrome of severe preeclampsia, and two or one clinical sign of HELLP at the same time (ELLP, EL or LP) [41]. In these classifications, liver enzymes and LDH levels are only considered elevated, when reaching a value over double the accordant highest normal

levels. As listed in table 1.5, the risk of severe maternal morbidity rises when laboratory values are elevated [41].

| Signs and symptoms | Laboratory values |
|---|---|
| Epigastric pain | Platelets $\leq 50000\ /\mu l$ |
| Nausea | Total serum LDH $\geq 1400\ IU/l$ |
| Vomiting | AST $\geq 150\ IU/l$ |
| Severe systolic hypertention | ALT $\geq 100\ IU/l$ |
| Severe diastolic hypertention | Uric acid $\geq 7.5\ mg/dl$ |
| Placental abruption | CPK $\geq 200\ UI/l$ |
| Eclampsia | Serum creatinine $\geq 1.0\ mg/dl$ |

Table 1.5: Admission risk factors for significant maternal morbidity/mortality HELLP syndrome [41]

### 1.4.2 Clinical presentation

The clinical presentation of HELLP syndrome is very variable in characteristics and intensity. Many patients may show rather nonspecific symptoms for instance malaise, nausea and vomiting [55][69]. These signs are also found in PE patients without HELLP and furthermore in many medical conditions (gastroenteritis, cholecystitis [30]). Due to this fact, differential diagnosis of HELLP has to include gastrointestinal, respiratory, hematologic and hepatologic syndromes [55]. In general clinical presentation of HELLP alternates between periods of remission and exacerbation and these fluctuations make it more difficult to diagnose [65]. In 82 - 85% mild hypertension is present in HELLP patients [55]. The most common clinical sign is epigastrical pain (65% [54]), usually located in the epigastric or right upper quadrant of the abdomen. Second most frequent symptomes are nausea and vomiting with 36% occurrence. Right below with 31%, headache is observed in HELLP patients. Bleeding and visual changes are developed in 9% and respectively 10% of cases. Symptomes like jaundice (5%), diarrhea (5%) and shoulder or neck pain (5%) occur equally frequent [54]. In most cases clinical presentation includes gastrointestinal symptoms. Severe epigastric pain though seems to be an indicator for rapidly progressing HELLP [41].

### 1.4.3 Differencial Diagnosis

It is crucial to find the proper diagnosis, in order to initiate an accurate management and achieve the best possible outcome [56]. In table 1.6 the most important differential diagnoses are listed. In the following section only acute fatty liver of pregnancy is described in more detail, as it is the most common disease confounded with HELLP syndrome.

| Differential Diagnoses | |
|---|---|
| Acute fatty liver of pregnancy (AFLP) | Idiopathic thrombocytopenic purpura (ITP) |
| Hemolytic uremic syndrome (HUS) | Immune thromocytopenic purpura (ITP) |
| Systemic lupus erythematosus (SLE) | Antiphospholipid syndrome (APLS) |
| Hemorrhagic or septic shock | Fulminant viral hepatitis |
| Acute pancreatitis | Disseminated herpes simplex |
| Cholecystitis | |

Table 1.6: Differential Diagnosis of HELLP [55]

#### Acute fatty liver of pregnancy

AFLP is very similar to HELLP and is therefore discussed as example for the difficulty of differential diagnosis. Difficulty to distinguish between AFLP and HELLP is due to the fact that their clinical signs and even laboratory findings are very similar. Onset of disease is between the $27^{th}$ and $40^{th}$ week in some cases postpartum, representing the same onset of HELLP itself. Symptoms like malaise, nausea, vomiting, epigastric or right upper quadrant pain, headache and jaundice are possible, but at the same time not inevitable in 15 - 20% of cases. Low-grade fever, as well as, hypertension, proteinuria and bleeding were observed in some patients, making it exceedingly difficult to differentiate between AFLP and HELLP. Only a liver biopsy would be sensitive enough to confirm diagnosis, in contrast to a CT scan or ultrasonography of the liver. However in clinical practice this procedure does not bear any meaning [56]. Laboratory findings such as elevated direct bilirubin and prolongation of coagulation in combination with mild thrombocytopenia, suggest early states of AFLP, though much more than in HELLP syndrome [41][55].

Evidently, even very experienced obstetricians have trouble finding the right diagnosis [56]. One possibility to distinguish AFLP and HELLP could be the sFlt-1/PIGF ratio that Schoofs et al. found to predict PE. One would need to try to implement it on HELLP, see in subsection 1.3.2. Soon it will be possible to predict HELLP early in pregnancies

with certain biomarkers. Then clinical symptomes upon onset will not be the only criteria for diagnosis [53].

## 1.5 Maternal morbidity and mortality

Patients with HELLP face a high risk for mortality as well as for several different morbidities [41]. Different categories of morbidities are discussed below and will point out the extential defects in organ systems caused by HELLP.

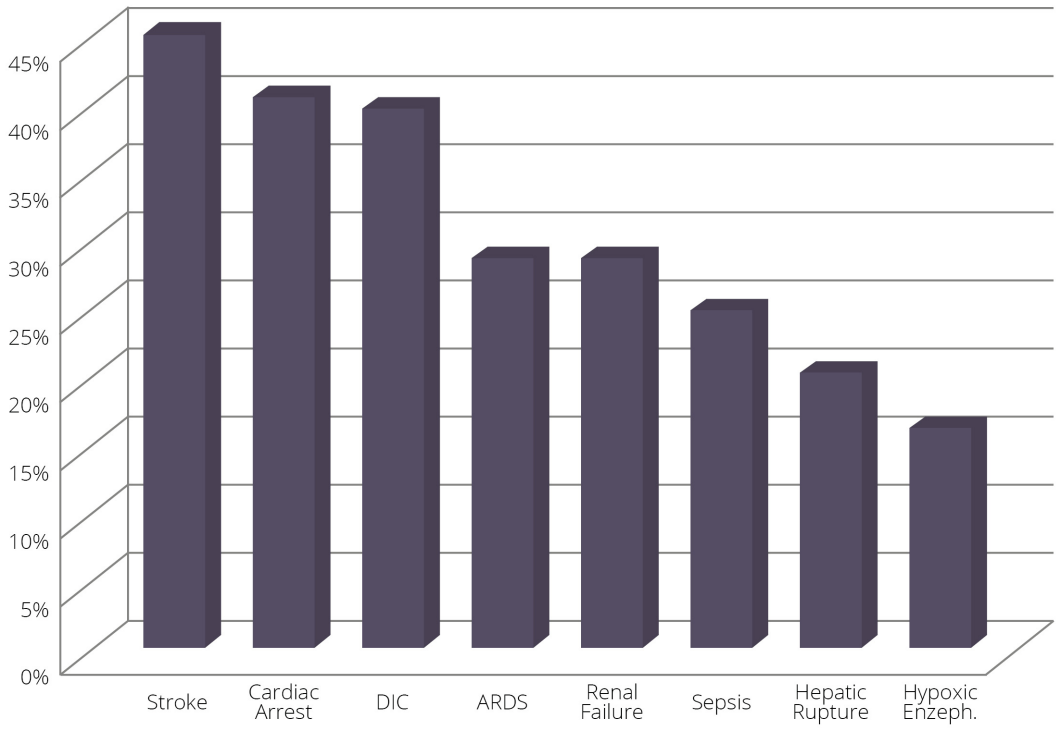


Figure 1.2: Contributing factors to deaths of women with HELLP in decreasing frequency [41]

### 1.5.1 Disseminated intravascular coagulation

For DIC, prevalence was found to be in 35 of 201 (17.4%) of patients with HELLP patients (class 1) in contrast to 0.5% in patients with non-HELLP severe preeclampsia.

In general it was observed that the incidence of clinically significant bleeding requiring transfusion therapy escalates with the severity of HELLP [41].

### 1.5.2 Liver rupture or Liver hemorrhage

The incidence of liver rupture or bleeding is only 1% of patients with class 1 HELLP (Tennessee classification). Interestingly all three cases of liver damage of any sort (subcapsular liver hematoma, liver hemorrhage, liver rupture) in the Mississippi series have been class 3 HELLP syndrome at the moment of first encounter of the complication [41].

### 1.5.3 Renal dysfunction

Patients with postpartum HELLP or placental abruption are more likely to suffer from renal complications such as renal insult. Renal morbidity gets more probable in patients with HELLP class 2 or even 1. Martin et al. found in their series, that acute renal failure was associated with Tennessee class 1 and 2 and all 4 patients with acute tubular necrosis had Mississippi HELLP class 1 [40][41].

### 1.5.4 Central nervous system

Suffering from class 1 or 2 HELLP syndrome without incidence of eclampsia, women are 3.5 times more likely to have severe central nervous morbitity than those with HELLP class 3 or severe preeclampsia alone. This means complications like stroke, coma and changes in mental state [41].

### 1.5.5 Cardiopulmonary system

Patients, who were diagnosed with class 1 HELLP have a 2.2 higher risk of developing a cadiopulmonary problem, such as pulmonary endema compared to those with severe preeclampsia alone [40][41].

### 1.5.6 Infection

Compared with women suffering from severe preeclampsia, HELLP patients more frequently get infectious morbidity (20% vs 43%). Amongst others three aspects have been

found to influence the incidence of infection in HELLP patients: corticosteroid application, delivery and transfusion. With corticosteroids involved in the treatment, the necessity for blood tranfusions decreases and consequently the occurrence of infection is reduced from 43% - 18%. The morbidity of infection is doubled by abdominal delivery from 19% - 41% [40][41].

### 1.5.7 Mortality

HELLP syndrome patients carry a higher risk for mortality in pregnancy (1%) [55]. As much as 45% of the occured deaths are in association with cerebral stroke or intracranial bleeding. Patients with class 1 HELLP are more likely to be affected by mortality, as they represent 60% of reported deaths from HELLP syndrome [41].

## 1.6 Neonatal outcome

The reason why perinatial mortality is so much higher in severe preeclampsia and HELLP pregnancies is mostly because of the need for delivery before term. Growth restriction and fetal distress were observed more often in pregnancies complicated with HELLP, as well as them being the other reasons for reported high mortality rate [41]. Perinatal mortality rate was between 7.4% and 20.4% in some series. As mentioned above, preterm delivery is very frequent in HELLP pregnancies (70%). It is associated with neonatal complications, such as bronchopulmonary dysplasia, intracerebral hemorrhage, necrotizing enterocolitis and respiratory distress syndrome [55]. The more severe HELLP gets, the higher are the rates of neonatal deaths and perinatal mortality. Albeit, outcome is probably more associated with gestational age at delivery than severity of disease [41].

## 1.7 Therapeutic concepts

As disease develops from the placenta, delivery is the causal therapy of HELLP syndrome. Facing HELLP onset earlier than $27^{th}$ gestational week, delivery is disadvantageous from the fetal point of view. It is only indicated when no additional danger is caused for the mother and the child [44][55]İt is important for patients that multilayered therapeutic concepts are applied, as HELLP is a systemic disorder affecting various organ systems. The main compounds of treatment are hyptertension control, iv. glucocorticoids and iv.

magnesium administration which might help stabilizing HELLP patients [43]. By close monitoring (intermediate care unit), controlling fluid balance and neurological check-ups deterioration of disease could be detected early.

Corticosteroid administration improves laboratory parameters and therefore could enhance maternal outcome [64][65][43]. As mentioned in subsection 1.5.4 central nervous morbidity is possible in very severe cases. Intravenous magnesium was found to be beneficial in preventing especially eclamptic seizures, see subsection 1.8 for more detail. On the other hand, administration of glucocorticoids can be used as a possibility to gain time to stabilize the patient and start fetal lung maturation for instance. As thoroughly explained in subsection 1.3.3 and 1.5.6, in HELLP patients inflammatory processes play an important part in disease development. The anti-inflammatory and immuno-suppressive effects of cortisone derivatives are conducive for stabilization and management of HELLP patients [41][55][68]. The main compounds of HELLP management like corticosteroids, magnesium and hypertensive therapy have been the subject of much research. Their basic characteristics and effects in HELLP patients are outlined in more detail below.

### 1.7.1 Magnesium sulfate

Intravenous infusion of magnesium sulfate is part of the management of HELLP syndrome and is mostly given for prophylaxis of eclamptic seizures. It is not known what mechanism is behind the effect of magnesium sulfate but it seems to have multiple mechanisms of action. Magnesium acts as a peripheral and cerebral vasodilator and therefore decreases the vascular resistance [68][16]. Is the blood-brain barrier disrupted due to hypertension, the risk of eclampsia associated brain endema is elevated. Magnesium sulfate serves as a protector of the blood-brain barrier through decreasing the permeability of the endothelium. It also has a central anticonvulsant effect [16]. A large randomized study showed reduction of eclampsia rate of 58% as well as a lower maternal mortality for women who received magnesium sufate [3].

### 1.7.2 Corticosteroids

Cortisol is an endogenous glucocorticoid, which is produced in the adrenal cortex and secreted amongst others in a circadian rhythm. A pituitary hormone called adrenocorticotropic hormone (ACTH) induces the production of cortisol (hydrocortisone) and other glucocorticoids, mineral corticoids and sex corticoids in the adrenal gland. This is reg-

| Magnesium doses | Dosage and serum levels |
|---|---|
| Loading dose | 6g iv. over 20-30 min |
| Maintenance dose | 2-3g iv. per hour |
| Recurrent seizures | Reload with 2g over 5-10 min |
| | and/or 250 mg of sodium amobarbital iv. |

Table 1.7: Magnesium sulfate dosages and serum levels [44]

ulated by a hypothalamic corticotropin-releasing hormone (CRH), as well as a negative feedback loop. High ACTH levels are caused by eating, light exposure or stress and consequently cortisol levels increase. Active cortisol is continuously converted into its inactive metabolite resulting in a plasma half-life of 60 to 90 minutes [62].

**Different cortisone derivatives**

There are several synthetic derivatives of glucocorticoids, some of which are short to medium-acting and some are long-acting glucocorticoids in terms of their pharmacological activity. Cortisol, prednisone and (methyl)prednisolone are counted to the former group with a biologic half-life of approx. 8 to 36 hours. Whereas, betamethasone and dexamethasone are assigned to long-acting glucocorticoids and have a biological activity of 36 to 54 hours. Prednisolone (half-life: 2-3 hours [46]) has five times the anti-inflammatory activity of hydrocortisone (pharmaceutic equivalent of cortisol). Dexamethasone and betamethasone on the other hand have 25 to 30 times more anti-inflammatory activity than hydrocortisone. Considering the consequences for the fetus, prednisolone is the better choice [62][65]. It does not pass the placental barrier in considerable concentrations and Runnard et al. therefore state that prednisolone should be used for maternal disease [63]. Whereas dexamethasone and betamethasone are indicated for primarily fetal treatment [62][65].

**Effect of corticosteroids**

The mechanism of action of corticosteroids in the body begins with binding to cytoplasmic glucocorticoid receptors (GRs) found in almost all human cells. This triggers transcription of proteins like anti-inflammatory protein and interleukin-1 receptor antagonist. At the same time GR activation can also lead to down-regulation of transcription of cytokines like IL-1 – 6, IL-11 – 13, interferon $\gamma$ and tumor necrosis factor $\alpha$ (TNF-

$\alpha$). The GRs inhibit transcription factors like AP-1 and NF$-\kappa B$. The latter are inducing inflammatory and immune response genes and are known to be very active in immune cells. Because of the influence on AP$-1$ and NF$-\kappa B$ glucocorticoids have a strong anti-inflammatory and immunosuppressive effect. They also block production of NO, arachidonic acid, ADP, collagen and thrombin causing the stabilization of endothelium and therefore decrease tissue endema and arrest platelet aggregation. Consequently, in the treatment of HELLP syndrome glucocorticoids are crucial as platelet aggregation is a problem as well as the systemic massive inflammatory response. Intravenous dexamethasone has shown to prevent disease progression and decrease maternal morbidity in HELLP syndrome patients [41][68]. Van Runnard et al., on the other hand used prednisolone as the corticosteroid of choice. They state that the SIRS-like presentation of HELLP justifies high-dose prednisolone, as it is also part of the treatment of anaphylaxis [65]. Its membrane stabilizing effect can prevent delayed anaphylactic reactions [18][50][68].

A crucial effect on the fetus has already been found by Liggins and Howie: Corticosteroids can induce lung maturation in pregnancies with impending preterm delivery [35]. The side-effects of long-term glucocorticoid administration are to be avoided by reducing the period and dose of treatment to a minimum. Long periods of cortisone treatment lead to osteoporosis, glucose intolerance, skin atrophy and eventually immune suppression [62]. Effects on the fetus have to be considered as well, as long-term or high-dose treatment can lead to fetal adrenal suppression and low birth weight [33].

## 1.8 Management

There are many different approaches of managing HELLP syndrome, even among well-published experts [6]. One of the most recognized way of managing HELLP syndrome is the Mississippi Protocol. It is outlined below as one possibility of HELLP management and used as inspiration for our own concept. The Mississippi protocol focuses on three major aspects such as [68]:

1. Administration of iv. dexamethasone
2. Administration of iv. magnesium sulfate
3. Blood pressure control

Firstly, magnesium sulfate is generally given at delivery and until 24 hours postpartum in order to minimize the risk for eclamptic seizures. Considering postpartum onset or facing

severe exacerbation of HELLP, the duration of iv. magnesium administration needs to be reevaluated [68]. It is suggested to continue magnesium therapy for an undefined time, unless HELLP is complicated by acute renal failure [7].

Secondly, blood pressure management involves iv. or oral application of anti-hypertensive medication. Drugs such as hydralazine or labetadol are to be considered aiming at systolic BP under 160 mmHg and diastolic BP under 100 mmHg [68]. German guidelines suggest the same threshold and urapidil for oral respectively nifedipin for iv. administration as first line of treatment for severe hypertension. In Europe dihydralazine is used instead of hydralazine but is known to have more side-effects (tachycardia, headache) than the other substances [15].

Thirdly, management of HELLP includes iv. dexamethasone treatment of 10 mg every 12 hours until platelet count is close to 100000 $\mu l$. At this point 5 mg dexamethasone are given twice with a period of 12 hours between administration to prevent recurrence of thrombocytopenia. It is vital to start iv. corticosteroid treatment as soon as HELLP class 1 or 2 is diagnosed. Furthermore HELLP class 3 or partial HELLP are dealt with accordingly if accompanied by eclampsia, severe systolic hypertension, heavy epigastric pain or a worsening clinical presentation [68]. Martin et al. found five aims of treatment which are met in the above described protocol [43]:

1. Arrest, Reverse, Shorten HELLP
2. Prevent HELLP progression to class 1
3. Prevent new maternal morbidity and prevent maternal mortality
4. Minimize perinatal morbidity and mortality
5. Undertake a timely delivery in an appropriate hospital setting

There is controversy about when delivery is indicated. Some claim that the diagnosis of HELLP is indication enough for quick delivery [69]. For term pregnancies (≥34 weeks) and pre-term pregnancies with signs of multiorgan failure this is most commonly the procedure of choice. Complications like DIC, liver hemorrhage, renal failure, placental abruption or fetal distress are some examples for a systemic problem in the mother. There is no consensus yet about how to proceed with pre-term pregnancies complicated by HELLP with neither acute exacerbation in the mother nor signs of fetal distress. Two

main pathways are found in literature: Induction of lung maturation with bethamethasone or prolongation of pregnancy until term is reached or systemic complications occur [22][36][48][54][55]. Martin et al. state that delivery should be timed between 24 and 72 hours after diagnosis of HELLP [43]. We used Martin et al's goals and the latter considerations as starting point for the development of our own treatment plan, which can be found in section 5.3.

# 2 Material and methodes

## 2.1 Recuitement and study population

The department of Gynecology and Obstetrics of the Medical University Graz is the biggest tertiary care center of the south-east of Austria with about 3000 deliveries per year. After approval from the local ethics committee (ethic committee of the Medical University Graz EK number: 25-407 ex 12/13) we analyzed the medical records of all obstetric patients who were admitted to the obstetrical policlinic at the University hospital of Graz. They were admitted between three weeks before to one week after birth from January 1$^{st}$ 2005 to December 31$^{st}$ 2012 with platelet count $\leq 100000/\mu l$. Informed consent was not necessary because of the retrospective study design and the anonymized data analysis. Initially 437 patients with thrombocytopenia were sorted out based on platelet counts of below $100000/\mu l$ at least once during their hospital stay. Of those 437, some 107 also had considerably elevated AST/ALT levels, that is more than double of the basic value and LDH values with over $265 IU/l$; all laboratory criteria present at the same time. Therefore they meet the criteria for HELLP syndrome defined by Sibai et al. and after modifications and based on the austrian and german guidelines. Eventually we analysed 80 of them, excluding those with insufficient data regarding their pregnancy and obstetrical history [55][15]:

**Hemolysis** $\uparrow$ LDH (depends on the laboratory)

**Elevated liver enzymes** $\uparrow$ ALT/AST (depends on the laboratory)

**Low platelets** $\downarrow$ Platelet count ($\leq 100000/\mu l$)

## 2.2 Groups

Three main groups were established:

**1 PRED group** treated by high dose prednisolone administered one to three times standard therapy regime ($n = 50, 62.5\%$)

**2 MIX group** received more than three doses and different types of corticosteroids ($n = 12, 15\%$)

**3 NO group** receiving no corticosteroids ($n = 18, 22.5\%$)

Most of the patients did get prednisolone according to the introduced therapeutical concept, hence this group (PRED) turned out to be the biggest with 50 patients. When the data was analysed, 18 patients were found who did not get any cortisone derivatives and these patients were admitted into no cortisone group (NO). MIX group only came into existance when the data was done being collected and documented. We found that some of our patients did not fit into PRED group because the therapeutical concept with prednisolone has not been applied, on the one hand. On the other hand these patients did get some cortisone iv. but not prednisolone. Whenever other types of glucocorticoids were used, like methylpredisolone or dexamethasone intravenously or even orally, these patients were put into MIX group. Independently from these groups, the cohort was distributed into Mg and NoMg, correlating with whether or not patients were given iv. magnesium sulfate during their hospitalization.

**Mg group** Patients with intravenous magnesiumsulfate infusion

**NoMg group** Patients without intravenous magnesiumsulfate infusion

The rate of occured eclampsia and neurological deficiency was determined during or after intravenous magnesium therapy and allows us to assert if magnesium sulfate transfusion is beneficial for HELLP patients.

## 2.3 Outcome measures

We analyzed these 80 patients firstly according to general, maternal and pregnancy associated data, like: age, body mass index (BMI), parity, gestational age at admittance, gestational age at delivery, delivery mode, antepartum or postpartum HELLP and maximum systolic and diastolic blood pressure (BP). Outcome was defined by these parameters:

– Time of hospitalization, frequency and time in the ICU

- Maternal complications/morbidities and necessity of special treatment (transfusions, dialysis, coagulation factor, ventilation)
- Maximum AST/ALT and LDH levels as well as minimum platelet counts
- Required medication
- Perinatal outcome

Perinatal outcome included the incidence of Asphyxia (NA-pH $\leq$ 7,00 or Apgar score after 5 min $\leq$ 7), completion of lung maturation and the occurance of antanatal HELLP. Additionally the hours of pregnancy prolongation were analysed here, as well.

## 2.4 Statistical Analysis

The effect of high dose prednisolone (0.5-1 g one to three times) on maternal and neonatal outcome in HELLP syndrome was analyzed in a retrospective study. Data was presented in absolute numbers respectively percentages. We compared normally distributed data using mean value/standard deviation and non normally distributed data using median with range (minimum-maximum). Statistic on qualitative data was performed using Chi square or Fisher's exact test with expected values $\leq$ 5. For normally distributed quantitative data independent T test and ANOVA calculation were conducted. The former for comparing group 1 (PRED) with group 3 (NO) and the latter for comparing all three groups. In that case output of results was as arithmetic mean and standard deviation. In the often occured case of non-normally distributed and therefore non-parametric data Mann-Whitney U test for two samples was conducted. For three samples Kruskal-Wallis test was used. Median and the minimum and maximum values were the results for that kind of data. A two-sided p-value of less than 0.05 was considered significant.

# 3 Results

## 3.1 Cortisone groups

### 3.1.1 Characteristics

The PRED group (group 1) consists of 50 patients who received 0.5 to 1 g prednisolone up to three times. Patients who did not receive any cortisone treatment were integrated in the NO cortisone group (group 3), consisting of 18 patients. 12 patients were assigned to MIX (group 2), who received more than 3 doses of cortisone and not only prednisolone but dexamethasone or methylprednisolone, as well. In the course of analysis MIX group data showed that many variables differ significantly from the other groups. This group will be characterized in more detail below.

| **Groups** | NO | PRED | MIX | p-value |
|---|---|---|---|---|
| Patients (n) | 12 (15.0%) | 50 (62.5%) | 18 (22.5%) | - |
| Mean Age y | 30.1 ± 5.7 | 30.9 ± 5.0 | 33.4 ± 6.0 | 0.240 |
| Nulliparity (n) | 13 (72.2%) | 41 (82.0%) | 10 (83.3%) | 0.814 |
| Antepartum HELLP (n) | 14 (77.8%) | 37 (74.0%) | 9 (75.0%) | 0.951 |
| Postpartum HELLP (n) | 4 (22.2%) | 13 (26.0%) | 3 (27.0%) | 0.951 |
| BMI | 28.1 (21-37) | 26.7 (21-48) | 26.1 (19-40) | 0.619 |
| Mean GA at admission wk | 34.8 ± 3.9 | 33.6 ± 4.9 | 28.9 ± 5.0 | 0.008 |
| Mean GA at delivery wk | 35.1 ± 3.8 | 33.9 ± 4.7 | 29.5 ± 4.5 | 0.007 |
| BP max systolic | 163.8 ± 19.1 | 169.6 ± 20.4 | 183.6 ± 19.1 | 0.055 |
| BP max diastolic | 99.4 ± 14.4 | 102.4 ± 10.5 | 112.5 ± 12.0 | 0.025 |

GA = Gesational age, BP = Blood pressure, BMI = Body mass index, ± = standard deviation

Table 3.1: Demographic data of cortisone groups

The established groups vary in numbers of patients as most of the patients were treated with the therapeutical concept of high-dose prednisolone iv. introduced into our department around 2007. Therefore PRED group contains most of the analysed patients. In most general aspects, like age, Body mass index (BMI), type of HELLP or nulliparity, the three groups are very similar. Already in the general maternal data MIX patients differ strongly from the other two groups (see table 3.1). MIX group is the smallest of the formed groups. Looking at the numbers in variable gestational age at admission for MIX patients, mean admission of patients is almost five weeks earlier than in the other two groups. Delivery happens almost 4 weeks earlier than in PRED or NO, suggesting an early onset of HELLP syndrome in these patients. The mean of maximal systolic blood pressure is at least some 14 mmHg higher. Diastolic maximal blood pressure on the other hand is significantly higher than in the other groups.

## 3.2 Outcome

Outcome of patients was defined by 5 aspects, which are analyzed indepentently for the sake of clarity.

### 3.2.1 Hospitalization

Patients in MIX group were tendentially (p=0.016) longer hospitalized than patients in PRED and NO. Other than that, their time and number of ICU stays did not significantly vary from group PRED or NO. Only hospilization time of MIX patients was significantly higher than in patients of PRED and NO (p=0.004).

| **Hospitalization** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| ICU (n) | 5 (27.8%) | 13 (26.0%) | 2 (16.7%) | 0.761 | 1.000 |
| ICU stay (d) | 1.9 (1.4-3.9) | 1.7 (0.5-23.5) | 3.0 (1.0-4.9) | 0.635 | 0.375 |
| Hospitalization time (d) | 7.8 (3-21) | 6.5 (2-42) | 14.0 (6-31) | 0.004 | 0.160 |

Table 3.2: Hospitalization

### 3.2.2 Complications

The following tables show results from analyzing occured complications and morbidities in HELLP patients from the three groups. Hemorrhagic complications were defined as

bleeding in brain, liver, kidney or because of uterine atony, placental abruption or revision. Bleeding in the kidneys was not found in any of our patients. In PRED two women suffered from intracranial hemorrhage and four from uterine atony. In MIX group one had an intracranial bleed and in NO one liver hematoma and one patient with uterine atony occured. Comparing PRED and NO non of the incidences of complications significantly varied in these two groups.

| **Morbidity and mortality** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Eclampsia (n) | 1 (5.6%) | 3 (6.0%) | 0 (0.0%) | 0.688 | 1.000 |
| Liver rupture (n) | 1 (5.6%) | 1 (2.0%) | 0 (0.0%) | 0.592 | 0.462 |
| Maternal mortality (n) | 0 (0.0%) | 1 (2.0%) | 0 (0.0%) | 0.738 | 1.000 |
| Pulmonary endema (n) | 1 (5.6%) | 1 (2.0%) | 0 (0.0%) | 0.592 | 0.462 |
| Renal failure (n) | 3 (16.7%) | 7 (14.0%) | 3 (25.0%) | 0.717 | 0.649 |

Table 3.3: Maternal morbidity and mortality

| **Intensive Care treatment** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Blood transfusion (n) | 3 (16.7%) | 5 (10.0%) | 6 (50.0%) | 0.005 | 0.428 |
| Catecholamines (n) | 0 (0.0%) | 5 (10.0%) | 1 (8.3%) | 0.383 | 0.315 |
| Coagulation factors (n) | 3 (16.7%) | 6 (12.0%) | 2 (16.7%) | 0.842 | 0.690 |
| Hemodialysis (n) | 0 (0.0%) | 1 (2.0%) | 0 (0.0%) | 0.738 | 1.000 |
| Platelet transfusion (n) | 3 (16.7%) | 9 (18.0%) | 5 (41.7%) | 0.171 | 1.000 |

Table 3.4: Intensive Care treatment

However, comparing all three groups we find a significance in the incidence of blood transfusions ($p = 0.005$). This can be ascribed to the high number of patients in MIX group needing blood transfusions (50.0%). With a p-value of 0.057 placental abruption occured almost significantly more often in MIX patients. Also a tendency was found in the incidence of platelet transfusions($p=0.171$). In MIX group five (41.7%) of patients had platelet transfusions, compared to nine (18.0%) in PRED and three (16.7%) in NO. Looking at the incidence and localization of hemorrhagic complications as defined below, we found no significance in neither analysis.

| **Hemorrhagic complications** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Occurance (n) | 2 (11.1%) | 6 (12.0%) | 1 (8.3%) | 0.937 | 0.645 |
| Brain (n) | 0 (0.0%) | 1 (2.0%) | 1 (8.3%) | 0.335 | 1.00 |
| Liver (n) | 1 (5.6%) | 1 (2.0%) | 0 (0.0%) | 0.592 | 0.462 |
| Revision (n) | 4 (22.2%) | 6 (12.0%) | 2 (16.7%) | 0.573 | 0.437 |
| Placental abruption (n) | 0 (0.0%) | 0 (0.0%) | 1 (8.3%) | 0.057 | - |
| Uterine atony (n) | 1 (5.6%) | 4 (8,0%) | 0 (0.0%) | 0.584 | 1.000 |

Table 3.5: Hemorrhagic complications

### 3.2.3 Laboratory differences

In order to establish an overview of the severity HELLP in the different groups, the four main laboratoy values were compared. The mean of the lowest platelet count of the patients in PRED, MIX and NO group and the median of the highest value of AST, ALT and LDH of every group were compiled. There were no significant distinctions found between the groups. Concering laboratory values HELLP severety did not decisively differ between the groups. Although the data shows that patients in the MIX group have lower minimal platlet count and higher maximum AST, ALT and LDH levels than the other groups.

| **Values** | NO | PRED | MIX | p | p (1vs3) |
|---|---|---|---|---|---|
| Min TC (/$\mu$l) | 59.2 ± 23.3 | 59.9 ± 19.9 | 51.7 ± 21.3 | 0.473 | 0.904 |
| Max AST (IU/l) | 194.0 (56-1032) | 260.5 (69-3051) | 498.5 (53-3029) | 0.394 | 0.221 |
| Max ALT (IU/l) | 212.5 (69-798) | 238.0 (66-2497) | 444.5 (64-1813) | 0.523 | 0.334 |
| Max LDH (IU/l) | 450.0 (318-1509) | 550.0 (246-3205) | 783.0 (274-3624) | 0.393 | 0.723 |

TC = Thrombocytes

Table 3.6: Laboratory results of cortisone groups

### 3.2.4 Medication

Urapidil (Ebrantil) is an $\alpha$-adrenoceptor antagonist and is used for hypertensive therapy in PE patients and pregnancy related hypertension under the guidelines for pregnancy hypertension of the german association of gynecology and obstetrics. Nifedipin (Adalat) is a calciumreceptor antagonist which is also used for hypertensive therapy and belongs to

| **Medication** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Urapidil iv. (n) | 3 (16.7%) | 14 (28.0%) | 8 (66.7%) | 0.011 | 0.527 |
| Nifedipin iv. (n) | 0 (0.0%) | 2 (4.0%) | 0 (0.0%) | 0.540 | 1.000 |
| Dihydralazin (n) | 3 (16.7%) | 5 (10.0%) | 1 (8.3%) | 0.701 | 0.428 |
| Labetalol (n) | 0 (0.0% | 3 (6.0%) | 2 (16.7%)) | 0.180 | 0.560 |
| Furosemid iv. (n) | 7 (38.9%) | 12 (24%) | 7 (58.3%) | 0.060 | 0.227 |

GA = Gesational age, BP = Blood pressure

Table 3.7: Medication in cortisone groups

the first-line treatment of pregnancy induced hypertension as well. Labetalol (Trandate) was also used but is more common in the U.S. [68]. Dihydralazine is called Nepresol in Austria is used for hypertension in PE patients [15]. The incidence of iv. blood pressure medication was analyzed because only in more severe cases we used syringe pumps or iv. boluses to control blood pressure. In mild cases oral blood pressure medication was sufficient, making iv. administration an indicator for severety of disease. As mentioned in subsection 1.8 it has more side-effects and was mostly used additionally if urapidil and nifedipin were not sufficient [15]. Only the frequency of iv. treatment was counted and no significant difference was found between the groups.

### 3.2.5 Perinatal outcome

For the variable of pregnancy prolongation we used only antenatal HELLP patients, which includes patients with onset of disease of $\leq$ 34 weeks. Percentage of lung maturation was highest in MIX group in contrast to the other groups (p=0.014). All of the MIX patients had a cesarean section (p=0.143). Pregnancy prolongation was significantly longer than in PRED and NO groups with a p-value of 0.047. As were the number of antenatal HELLP syndrome in MIX compared to the other groups (p=0.038). The variable asphyxia was defined by APGAR score after five minutes below seven or pH value (umbilical artery) below 7.00 but it showed no difference between the groups.

| **Perinatal outcome** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Antenatal HELLP* | 7 (38.9%) | 23 (46.0%) | 10 (83.9%) | 0.038 | 0.602 |
| Asphyxia | 1 (5.6%) | 3 (6.0%) | 2 (16.7%) | 0.424 | 1.00 |
| Cesarean section (n) | 16 (88.9%) | 39 (78%) | 12 (100%) | 0.143 | 0.219 |
| Completed lung maturation course* | 3 (16.7%) | 15 (30.0%) | 8 (66.7%) | 0.014 | 0.385 |
| Fetal mortality | 0 (0.0%) | 3 (6.0%) | 1 (8.3%) | 0.513 | 0.391 |
| Pregnancy prolongation (d) | 0.8 (0.1-5.1) | 1.3 (0.0-24.7) | 4.9 (0.9-24.6) | 0.058 | 0.792 |

GA = Gestational age; * = for GA ≤ 34 weeks

Table 3.8: Perinatal outcome

## 3.3 Magnesium groups

### 3.3.1 Characteristics of magnesium groups

Even though WHO and the german gynecology and obstetrics guidelines recommend iv. magnesium sulfate at diagnosis of HELLP or severe preeclampsia, 26 of our patients did not receive any iv. magnesium therapy [15]. That allowed this study to form the control group (NoMg) for the magnesium group (Mg). Additionally oral intake of magnesium was analysed as well. We found that from NoMg patients, 21 did not even take magnesium orally during their stay. Almost every eclampsia observed, happend to patients in that sub group. More detailed evaluation, see in subsection 4.8.

| **Magnesium groups** | Mg | NoMg | p-value |
|---|---|---|---|
| Patients n(%) | 54 (67.5) | 26 (32.5) | - |
| Mean Age y | 31.1 ± 5.3 | 31.1 ± 5.6 | 0.989 |
| Nulliparity n(%) | 44 (81.5) | 20 (76.9) | 0.738 |
| Antepartum HELLP n(%) | 41 (75.9) | 19 (73.1) | 0.789 |
| Postpartum HELLP n(%) | 13 (24.1) | 7 (26.9) | 0.789 |
| BMI | 27.6 (19-48) | 25.9 (22-36) | 0.320 |
| Mean GA at admission (wk) | 31.7 ± 5.0 | 36.0 ± 4.0 | 0.000 |
| Mean GA at delivery (wk) | 32.4 ± 4.6 | 36.1 ± 3.9 | 0.000 |
| BP max systolic | 175.6 ± 19.8 | 159.5 ± 18.1 | 0.001 |
| BP max diastolic | 110.0 (73-142) | 96.5 (70-116) | 0.000 |

GA = Gesational age, BP = Blood pressure,BMI = Body mass index, ± = standard deviation

Table 3.9: Demographic data of magnesium groups

### 3.3.2 Results for magnesium-group versus no-magnesium-group

Our analysis showed that the difference in hospitalization time in NoMg patients and Mg patients were significant, hence women in NoMg had to stay less long. We confirmed that even in small sample size studies such as this one, eclampsia rate is almost significantly lower in Mg than in NoMg (p=0.098). There was no difference found between Mg and NoMg concerning the other variables, see in table 3.10.

| **Results** | Mg | NoMg | p-value |
|---|---|---|---|
| Eclampsia (n) | 1 (1.9%) | 3 (11.5%) | 0.098 |
| Hospitalization time (d) | 7.8 (3.3-42.2) | 6.3 (1.5-40.9) | 0.035 |
| Neurological symptoms (n) | 2 (3.7%) | 2 (7.7%) | 0.592 |
| Remaining neurocognitive deficit (n) | 1 (1.9%) | 2 (7.7%) | 0.245 |
| Sight disorder (n) | 8 (14.8%) | 3 (11.5%) | 1.000 |

Table 3.10: Results for magnesium groups

# 4 Discussion

In several randomized studies researchers found that the use of corticosteroids in management of HELLP syndrome is beneficial for outcome and the course of specific laboratory values [41][62][65]. Runnard et al., as one of the few, used Prednisolone as the glucocorticoid of choice but most researchers use iv. dexamethasone [65][43]. However, in our study prednisolone was used for the cortisone group (PRED). Whenever other types of glucocorticoids were administered like methylpredisolone or dexamethasone, patients were taken out of the cortisone group into MIX group. This was done in order to maintain a general conspectus over how much and what type of glucocorticoid is in the maternal system in our main group PRED. In order to tell if prednisolone is the effecious drug this group was compared to the no cortisone group.

## 4.1 First considerations

We were trying to show a beneficial effect of prednisolone in our small retrospective study. We first started by looking at the dynamics of every patients laboratory data and how the values would be affected by delivery and iv. prednisolone. This statistical analysis brought no significant results. The informative value of dynamics in lab work is almost exclusively dependent on the moment of blood draw. Also, defining a reasonable cut-off for pathological dynamic has shown to be difficult. That means we had difficulties determining how fast platelets should increase or ALT/AST/LDH should normalize in order for the results to make sense. Hence, in a retrospective study several problems occur that falsify results. The timing of delivery for instance, which is often followed by a fast normalization of HELLP specific laboratory values. HELLP syndrome usually progresses with a strong fluctuation of symptomes and laboratory values, as explained in subsection 1.4.2. In some cases we encoutered remission periods without changing anything in the treatment. However, some patients worsened even though they received the full spectrum of treatment.

Partial HELLP, post- or antepartum HELLP as well as early onset HELLP are different forms of the syndrome. They present differently in lab works and have various courses of disease. Partial HELLP may not appear as severe in terms of HELLP laboratory values but is complicated in 10% of cases with eclampsia or placental abruption [49]. Early onset HELLP is often associated with severe and fast deterioration of affected women [1]. In postpartum HELLP cases, remission is expected as the dysfunctional placenta is delivered and management is not complicated by fetal considerations [13]. But these women are at higher risk of developing renal complications [62][41]. The timing of drawing blood and interpretation of results in particular in the aforementioned cases would have to be adjusted accordingly. For instance, surveillance and laboratory controls for partial HELLP patients in case of possible worsening to full HELLP syndrome [38]. Additionally early onset cases need more close monitoring of the fetus and the mother if major complications occur. Only a prospective study could make adjustments like that possible. The results on laboratory dynamics were therefore not included. The mean of minimal platelets and of maximum ALT/AST and LDH values were only analyzed to determine severety of HELLP.

## 4.2 Prednisolone

Prednisolone is thought to be beneficial for women with HELLP. The syndrome is considered to have similarities with anaphylaxis or SIRS [41]. Dexamethasone and betamethasone are used for fetal indications like lung maturation because their active forms reach the fetal blood stream [65]. As explained in subsubsection 1.7.2, no active prednisolone crosses the fetal placental barrier in significant concentrations. It is therefore not affecting the child as much as dexamethasone and betamethasone. The latter is used to induce lung maturation in pre-term pregnancies ($\leq$ 34 gestational week) [55]. Van Runnard et al. claim the beneficial effect of prednisolone on HELLP patients. They showed in particular that it prevents the increase of cytokine IL-6 during HELLP exacerbation. They attributed this to the stabilizing effect of prednisolone on the endothelium [65]. Other studies claim that it increases platelet count [31]. However, they could not prove any effect of prednisolone on CRP or ALT/AST levels [65].

## 4.3 Magnesium sulfate

Intravenous magnesium sulfate over a syringe pump is part of the management of HELLP and severe preeclampsia since the beginning of the 20th century [20]. Even the WHO recommends usage of it in their guidelines for prevention and treatment of preeclampsia and eclampsia. As mentioned in subsection 1.7.1 the course of action is not fully understood yet but nonetheless clinical trials emphasize the importance of it as prophylaxis for eclampsia [16][68]. In our study we wanted to show this effect in a small number of patients.

## 4.4 Patient characteristics

All our patients were analysed for any risk factors for PE and HELLP. We included those for PE for the sake of completeness and because there are not many risk factors in our patients that are known to be only applicable for HELLP, see table 4.1.

| **Risk factors** | NO | PRED | MIX | p | p (1 vs 3) |
|---|---|---|---|---|---|
| Adipositas | 2 (11.1%) | 4 (8.0%) | 1 (8.3%) | 0.921 | 0.506 |
| Age ≥ 40 y | 0 (0.0%) | 1 (2.0%) | 0 (0.0%) | 0.738 | 0.735 |
| APLS | 0 (0.0%) | 1 (2.0%) | 1 (8.3%) | 0.335 | 0.735 |
| DM | 1 (5.6%) | 2 (4.0%) | 1 (8.3%) | 0.820 | 0.609 |
| MTHFR deficiency | 2 (11.1%) | 2 (4.0%) | 2 (16.7%) | 0.263 | 0.284 |
| Nulliparity | 12 (66.7%) | 37 (74.0%) | 9 (75.0%) | 0.818 | 0.379 |
| SLE | 0 (0.0%) | 0 (0.0%) | 1 (8.3%) | 0.057 | - |
| Patients with 2 risk factors | 2 (11.1%) | 5 (10.0%) | 2 (16.7%) | 0.806 | 0.602 |
| Patients with 3 risk factors | 0 (0.0%) | 1 (2.0%) | 1 (8.3%) | 0.335 | 0.735 |
| Patients with 4 risk factors | 1 (5.6%) | 0 (0.0%) | 0 (0.0%) | 0.175 | 0.265 |

APLS = Antiphospholipid syndrome; DM = Diabetes mellitus; MTHFR = Methylenetetrafolate reductase; SLE = systemic lupus erythematosus

Table 4.1: Distribution of risk factors among our patients

Risk factor analysis showed that risk factors among our patients are equally distributed. No group has significantly more women with risk factors. Autoimmune diseases which are known to create a higher risk for HELLP were found only in PRED and MIX. In particular APLS is associated with early onset HELLP and is found in MIX group which

contains significantly more antenatal HELLP cases whereas five of them were early onset HELLP syndrome [1]. APLS is known to further complicate course of disease and is probably the reason why this one patient was put in MIX [32].

One of our PRED patients suffered a sudden onset of catastrophic postpartum HELLP. Even though everything was undertaken to control the exacerbation, we lost her on the second day postpartum. The 33 year old bipara came to us as a term pregnancy in the $40^{th}$ week of gestation with beginning contractions. Inicially she presented with no right upper quadrant pain, normal blood pressure and no thrombocytopenia in her lab work. She delivered a female baby an hour later without complications. Two hours after delivery though, she showed signs of beginning HELLP with mild thrombocytopenia (109 /$\mu$l) and AST/ALT and LDH values of 251/223 IU/l and 575 IU/l respectively. At that point 250 mg of Prednisolone was administered and she was transferred to the ICU immediately. Her clinical status was deteriorating quickly, as only one hour later liver enzymes reached the the threshold of 2000 IU/l and LDH was measured with over 3000 IU/l. She developed hypertension, nausea, vomiting and uterine atony. The latter resulted in excessive bleeding and was controlled with iv. sulproston and dilatation/curettage. Soon after the unterine bleeding ceased she developed disseminated intravascular coagulation because of liver failure and uterine bleeding. She was also treated with iv. platelet/blood-/albumine transfusions and coagulation factors, such as fibrinogen, antithrombin and prothrombin. Nonetheless, after two days of ICU treatment she died from massive intracranial bleeding accompanied by an eclamptic seizure and subsequently fatal brain endema. Most researchers found that postpartum HELLP will resolve itself after 48 hours after delivery. But just like in this case patients with severe complications like DIC, acute renal failure or thrombocytes under 20000 /$\mu$l are likely to develop a deterioration of their clinical status [55]. This patient had no known risk factors neither for HELLP nor for PE and showed no signs of preeclampsia on admission. That was probably one reason she did not receive magnesium sulfate and subsequently lead to her eclampsia. Measures like plasma exchange could have been considered but at no time the patient was stable enough for it [38][37]. It was impossible to anticipate the course of disease in this woman but everything medically possible was conducted.

## 4.5 Group characteristics

The different-sized groups result from the fact that this study was conducted in retrospective. HELLP patients at the obstetrics university clinic of Graz from 2005 to 2013 were

included. A new concept of treatment was introduced around 2006 and was administered to most of the patients. Hence, the biggest group is PRED with 62.5% of patients, who received high-dosed prednisolone.

PRED and NO groups were compared and analysed in particular, as the MIX groups took a different stand in terms of comparability. Comparing all three groups at once produced more significant results than when comparing only group 1 and 3. The main reason is that MIX group is very heterogenous, containing many women with severe comorbidities, risk factors and strongly varying glucocorticoid intake. Further characteristics and analysis of MIX group can be found in table 4.5. Apart from cortisone groups, we formed two groups on account of magnesium sulfate administration, magnesium and no magnesium group.

Judging by the demographic data, patients in PRED are very similar to patients in NO. Mean age, parity, BMI and incidence of antepartum and postpartum HELLP is fairly similar to NO patients. Analysis of disease related variables like gestational age at admission and delivery, as well as mean systolic and diastolic blood pressure makes PRED and NO groups out to be very comparable. The only difference is PRED being the biggest of the three groups, consisting of 50 patients making NO too small to be used as control group.

The MIX group stands out from the other groups in almost every variable that was analysed. If p-value of all three groups showed a tendency or a significant difference it was exclusively because of strongly variant values in MIX group. This group was established due to the fact that some patients were given various cortisone derivatives both intravenously and orally. Sometimes patients received dexamethasone (10-12 mg) orally, following newly published studies. One received corticosteroids because of her severe migraine attack, as corticosteroids can act as emergency medication [19]. In another case we tried to prolongate an extremely early pregnancy (GA at admission 23.3) with IUGR with methylprednisolone, to maintain her pregnancy past the $24^{th}$ week. Methylprednisolone was given iv. and orally with very small doses (25 mg) to one MIX patient admitted to the ICU. Supposedly it was administered because of signs of SIRS or sepsis according to a study from 2003 [29], a measure that is now obsolete [58]. Most times it is not retraceable why patients of MIX received certain glucocorticoid derivatives. It mainly depended on the experience of the obstetrician on call, what type of derivative was given in which concentration.

| ID | Cortisone | Dose | GA | Grav/Para | Prol. (d) | Risk factors | Complications |
|---|---|---|---|---|---|---|---|
| 21 | Prednisolone | 15 x 12.5-100 mg | 24.7 | 1/0 | 12.5 | MTHFR deficiency<br>Placental insufficiency | Early onset HELLP, IUGR, |
| 29 | Oral dexamethasone | 1 x 20 mg | 32.9 | 1/0 | 9.5 | - | Placental abruption, Proteinuria |
| 53 | Oral methylprednisolone<br>Prednisolone | 1x 40 mg<br>5 x 0.25-1 g | 25.0 | 3/2 | 4.9 | SLE | Early onset HELLP, Proteiuria |
| 55 | Oral methylprednisolone<br>Prednisolone | 1 x 40 mg<br>1 x 0.5 | 27.0 | 1/0 | 24.6 | - | Early onset HELLP, IUGR,<br>Proteinuria |
| 60 | Prednisolone | 1 x 2.5 mg | 36.9 | 1/0 | 0.1 | - | - |
| 61 | Oral prednisolone<br>Prednisolone | 3 x 5-15 mg<br>7 x 25-500 mg | 24.6 | 2/1 | 0.1 | - | Early onset HELLP, IUGR |
| 70 | Oral methylprednisolone<br>Prednisolone | 1 x 40 mg<br>3 x 500 mg | 25.0 | 1/0 | 3.0 | - | Early onset HELLP, IUGR,<br>Proteinuria |
| 71 | Oral methylprednisolone<br>Prednisolone | 6 x 20-40 mg<br>1 x 1 g | 30.0 | 1/0 | 0.9 | Adipositas, MTHFR<br>deficiency | Proteinuria |
| 79 | Oral prednisolone<br>Prednisolone | 1 x 12 mg<br>3 x 0.025-1 g | 37.3 | 1/0 | 0.1 | Age ≥ 40 y, DM | Acute kidney failure,<br>Intracerebral bleeding, Proteinuria |
| 83 | Prednisolone three times<br>Oral dexamethasone | 3 x 0.5-1g<br>2 x 10 mg | 29.1 | 1/0 | 2.5 | - | - |
| 87 | Prednisolone | 2 x 100 mg | 31.0 | 1/0 | 4.4 | - | Proteinuria |
| 91 | Oral prednisolone<br>Prednisolone | 8 x 5-10 mg<br>1 x 1 g | 31.1 | 2/0 | 1.9 | APLS | IUGR, Proteinuria |

Prol. = Prolongation; MTHFR = Methylenetetrahydrofolate reductase; IUGR = Intrauterine growth restriction; DM = Diabetes mellitus;

SLE = Systemic lupus erythematosus; APLS = Antiphospholidpid syndrome

Table 4.2: Special features of MIX patients

When we first started collecting data, we expected to find a small number of HELLP patients without cortisone treatment. Finally we found 18 patients who didn't receive any cortisone as treatment for HELLP syndrome in the analyzed period of time. Three patients of NO received full lung maturation regimen (Betamethasone 12 mg twice at 0h and 24h) though, which is indicated in HELLP patients diagnosed before 34 weeks of gestation [55]. Another two patients from the NO group received one dose of betamethasone. We wondered why these patients were not treated with any glucocorticoids for maternal purposes. At first it seemed that these patients may have been at the clinic before the concept was introduced. But that was not the case as half of NO patients were admitted after 2007. One of the reasons is probably that HELLP was not diagnosed at time of admission. One of the clues is that significant laboratory values for HELLP were not drawn every day in most NO patients. The data suggests that most of NO patients only fulfilled the three HELLP criterias for one day or two and showed almost no clinical symptoms. Therefore no further diagnostic procedures have seemed to be necessary. Some NO patients with severe thrombocytopenia were treated with platelet transfusions. Liver enzymes were around 80 IU/l, suggesting that the liver was not affected in NO group as much as in other patients in MIX or PRED. Few patients in NO had very short hospitalization time.

Administration of magnesium sulfate plays an important part in eclampsia prophylaxis [68][55][49]. Still we included 26 HELLP patients between 2005 and 2013 who did not receive any intravenous magnesium. The magnesium group is much bigger and consists of 54 patients. Not only in size these two groups were found to be very different. Looking at mean gestational age at admission and delivery, NoMg patients significantly showed later onsets of disease (p value=0.000/0.000). The difference of mean blood pressures between magnesium and no magnesium group was also statistically significant (p=0.001/0.000), whereas systolic and diastolic values of Mg patients exceed the ones of NoMg women. Therein could lie one cause for the missing administration of magnesium in NoMg patients. The severety of HELLP syndrome could have been underestimated or HELLP was not recognized at all because of only mild hypertension present in NoMg patients. We assumed that early cases would be the ones who did not receive magnesium but most NoMg patients were included in the study from 2009. More clues are dicussed in subsection 4.8.

## 4.6 Differences between prednisolone group and no prednisolone group

In PRED we found a tendency of shorter time at the ICU (p=0.114), as well as shorter hospitalization time postpartum (p=0.160). From this it follows that in PRED patients remission is possible faster as the glucocorticoid lessened the inflammation process in the mother [62]. Lung maturation course was completed more often in PRED than in NO (p=0.391). Through the application of high-dose prednisolone the inflammatory process in HELLP was decreased and the endothelium could be stabilized [65]. This would explain why patients in PRED were stable enough to have a vaginal delivery as it created more time for induction of labor. Because the pregnancy was prolongated there was also more time to complete the course of lung maturation over 24 hours. Tendencially less percentage of women in PRED had to get a cesarean section than in NO, the result having a p-value of 0.158. In conclusion prednisolone improves fetal outcome as lung maturation could be finished [41], as well as decreses the risk of maternal morbidity through cesarean section [40]. The statistics mentioned above show a tendency toward a benefit for patients treated with PRED, a finding which is reinforced by several studies[65][64].

In this study we did not find any difference in the severity of thrombocytopenia, see in subsection 3.2.3. We also analysed the change of platelet count in dependency of cortisone administration. The results were not conclusive and showed no significants whatsoever which is why we did include them, see also in section 4.1. Administration of dexamethasone, however was found to have a significant effect on increasing platelet count [71]. The same could be shown in a prospective study with prednisolone[62], on the basis of glucocorticoids could inhibit the aggregation of platelets [26]. Many studys with dexamethasone have been performed and have shown benefits in the outcome of women with HELLP [38][39][42][49][57][55]. Like Van Runnard et al., we believe that the same is true for high-dose treatment with prednisolone [62][65][64]. There are some studies that could not show any effect of corticosteroids on outcome in HELLP patients [27][28][17][66][12]. But some of their studies have aready been confuted and shown to have flaws in their analysis of data [61].

There were some problems with this control group that could explain the missing significant results. In retrospective we only found 18 patients for NO, which makes it not suited to be comparable to the 50 patients in PRED. Another important question was whether some patients in NO really had HELLP syndrome. Their missing clinical symptoms and

liver enzymes only partially elevated up to 80 $IU/l$ are hints that might suggest at least a mild form of HELLP. This group therefore is not comparable in variables like maternal outcome and morbidity with the severe forms found in PRED and MIX groups. In mild HELLP syndrome (Mississippi classification 3) maternal morbidity is much less severe and outcome in general is better than in patients with severe HELLP syndrome [41][62]. At last, in NO group we did not exclude patients who received betamethasone. Seven patients in NO at least received one part of the regimen which could have tampered the results. As Martin et al stated that a control group where patients received cortisone for lung maturation purposes could lead to falsified and less significant results [43].

## 4.7 Differences between all three groups

### 4.7.1 Significant findings

The data shows that patients in MIX group were significantly (p=0.004) longer hospitalized than patients in PRED and NO. The difference being greater between PRED and MIX than NO and MIX. This is ascribed to the fact that mean gestational age at admission in MIX was 28.9 weeks, making MIX the group with the most antenatal HELLP patients (p=0.038). The main goal for these patients was to delay delivery and induce lung maturation to reduce fetal complication. That is why pregnancy prolongation was significantly longer than in PRED and NO groups with p=0.047. Even then, mean gestational age at delivery was significantly lower in comparison to PRED and NO (p=0.007). The percentage of lung maturation completion was the highest of all the groups (p=0.014), as MIX consisted of much more pre-term pregnancies. Reviewing the MIX patients for a second time showed that 5 of 12 patients met the criteria for early onset HELLP syndrome, which is associated with a severe form of the disease and fetal growth restriction [1][41]. In three out of the five early onset patients we found fetal growth restriction (FGR), causing a higher risk for the newborns of a worse outcome [2][51]. Furthermore systolic and dyastolic mean blood pressures in that group were significantly higher (p=0.055/0.025) than in PRED and NO, suggesting a severe form of disease. The following significant findings also support the assumption that MIX patients suffered from a severe form of HELLP syndrome: Patients in MIX needed more blood transfusions (p=0.005) and more of them were given iv. uradipil for high blood pressure treatment (p=0.011).

Apart from the difference in iv. uradipil treatment, no difference was found in blood pressure medication regarding all three groups and when comparing PRED and NO. But in

this study only the frequency of iv. administration was analyzed and not the overall dose every patient eventually recieved. This parameter would be more suitable to determine the severety of hypertension and associated with that the severity of HELLP syndrome in these patients.

### 4.7.2 Other findings

Starting with the demographic data of the three groups, MIX group consists of women with an average age of 33.4 years. That is the highest mean age of all the groups, the difference though not being statistically significant. In variables like parity, BMI and incidence of antepartum and respectively postpartum HELLP the MIX group does not stand out. As mentioned in 4.7.1 MIX group might consist of patients with a severe and complicated form of HELLP. The results confirm that as women in MIX had higher incidence of bleeding (p=0.067). Tendentially MIX patients have gotten more furosemid (p=0.060) and platelet transfusions (p=0.171) than the women of the other groups. All of MIX patients had a cesarean section, the p-value being 0.143 compared to cesarean section incidences in PRED and NO.

There were no differences found in laboratory values of all three groups. As decribed in subsection 3.2.3 MIX group patients stand out slightly again but not statistically comprehensible. It is questionable whether the mean of the lowest platelet value and the mean of the highest value of ALT, AST and LDH was the right variable to use to determine severety of HELLP. Looking at the hospital course many patients with severe disease showed very low platelet counts/high ALT,AST,LDH for a longer period of time than the ones with mild HELLP. This is not reflected in the aforementioned variables. The differences might be more distinct if the dynamic of these laboratory values were analysed over the course of hospitalization. In order to do so, one would have to draw blood at a certain point of time. For example half an hour before predisolone admission and 6 hours after, to be able to see what effect the drug might have had.

To sum up, course of disease in MIX patients was less predictable, they were more likely to suffer from major complications. Furthermore they were more inclined to fetal complications due to low gestational age [2][51]. Hence, the significantly longer prolongation time MIX patients were more likely to suffer from HELLP for a longer period of time. That could have lead to a more severe clinical picture in them as HELLP had time to worsen. Most patients even with early onset of HELLP should be delivered within

a week [55]. It seems that the early onset and resulting severety of HELLP lead to the application of different therapeutical concepts[1].

## 4.8 Differences between magnesium and no magnesium groups

We found a significant difference (p=0.035) in hospitalization time between Mg and NoMg patients. As women in NoMg were admitted significantly shorter than the ones in Mg group, we concluded that they might have had a mild form of HELLP. This could have led the attending obstetrician to not administer iv. magnesium sulfate. The main focus was on the incidence of eclampsia in magnesium and no magnesium group. One patient in the former group suffered from an eclamptic seizure and therefore only a slight improvement of eclampsia occurances could be shown between these two groups (p=0.098). The course of disease of this particular patient was analysed in more detail. We found that the eclampsia occured almost 10 hours after weaning the magnesium sulfate syringe pump. Right after the magnesium administration was resumed and no other eclamptic incident was documented therafer. Therefore intravenous magnesium sulfate does prove to be beneficial even in this small-sample study and should be continually be used for prophylaxis in HELLP and PE patients.

## 4.9 Controversies with this study

In a retrospective study one can only work with the existing patients and assign them to certain groups. Our main group was PRED, where patients received a certain amount of prednisolone. But we also found some that did not get prednisolone, hence we formed a NO group and used it as our control group. But there were some patients though, who did not fit into any of the two groups. We saw that what therapeutic concept the patients were treated with, varied much with the physician on the case. Some were familiar with the therapeutic concept that should be executed upon HELLP diagnosis and some were not. Therefore, standard protocol was not used on all HELLP patients as it should have been. That lead to some cases, where patients received a mixture of different cortisone derivatives orally and intravenously. These particular patients were put into the MIX group, as the spectrum of corticosteroids used were very diverse even within the group itself. Accordingly the comparablilty of MIX with the other two groups was limited.

In this study the demographic characteristics of NO group were matchable to the ones of PRED patients. Its small size though in comparison to PRED prevents it from serving as a proper control group for PRED. Furthermore patients who had received lung maturation were not excluded from NO or at least put into MIX group.

In subsection 1.4.3 the difficulty of delimiting HELLP from clinically similar syndromes and diseases is illustrated. In retrospective it is almost impossible to be sure whether some of our more complex patients were suffering from HELLP syndrome or rather from fatty liver of pregnancy or lupus erythematosus exacerbation. This might also be related to the diverse and incomplete documentation found in some files. It prevented us from retracing the exact time scale of events and medication administration. Some notes of gynecologists and nurses even contradicted themselves. Handwritten documentation sometimes followed no specific regimen and did not illustrate clinical presentation over the course of hospitalization. In many cases important laboratory values were not ordered or patients were released too early and therefore could not have gotten a proper follow-up. Some of NO patients therefore could not have been included into the study because of lack of laboratory and clinical data. Course of disease differed very much between antepartum and postpartum HELLP. Hence, postpartum and antepartum studies should not be compared because PE and HELLP always ceases after delivery of the placenta [62]. The effect of high-dose cortisone on the fetus has not been evaluated in this study but needs to be considered when creating a regimen. Findings suggest that long-term high-dose corticosteroid tretment has a negative effect on fetal outcome [33].

Finally, during the time frame of the study LDH was used as a parameter for hemolysis. Recent studies show that LDH is not the best laboratory value to do so. As it turns out only two of the five subforms of LDH increase because of hemolysis. Reduced serum haptoglobine is a better and more specific parameter for hemolysis and should rather be used for that [55].

# 5 Conclusion

The syndrome of hemolysis, elevated liver enzymes and low platelets is a severe complication of 0.2-0.8% of pregnancies leading to maternal and fetal morbidity and mortality[1][41]. This small retrospective study aimed to evaluate our multilayered therapeutical concept with high-dosed prednisolone as glucocorticoid of choice as well as develop a management plan for future HELLP patients.

## 5.1 Review of study

Around 60% of our HELLP patients received high-dose predisolone and were selected into PRED group. Patients in this group consistently received 0.5 to 1g upon HELLP diagnosis which made them a very homogenous group concerning cortisone administration. However, MIX group was established as these 12 patients did not fit into neither PRED nor NO group. The MIX group came into existance solely because of the retrospective design of the study and constisted of many high-risk and early onset HELLP patients. In future studies a third group might be formed from high-risk patients but without treating them with various groups and application forms of glucocorticoids. The fact that these patients received so many different corticosteroids prevented us from finding out which of the cortisone derivatives was in fact effective.

The collected data of the patients was extensive and provided information about group characteristics, HELLP severity and outcome parameters. Again, because the study was conducted in retrospective there was no way of analyzing the dynamics of laboratory parameters over the time of hospitalization. Laboratory analysis therefore was limited to mean minimum platelets and mean maxium ALT/AST and LDH. This data only suggests the severity of HELLP in the three groups but can not be used to find a beneficial effect of prednisolone. As several studies found this effect we are certain that study design, no suitable control group and no standard cortisone regimen was due to the missing significance

in our results [62][64][65]. Magnesium and no magnesium groups made perfect sense as iv. magnesium sulfate is established as recommended prophylaxis treatment in HELLP patients [15]. In our study we could show the importance of magnesium administration and how it needs to be part of a multilayered concept at all times through the course of HELLP syndrome.

## 5.2 Suggestions for future studies

For the future it would be beneficial to conduct a prospective study over 10 years, trying to collect at least 50 patients per group. With the results of this study, reasonable groups could be determined in advance and matching patients could be assigned to them. A time scale should be elaborated for lab collections, application of corticosteroids and clinical examination. There should be a focus on differential diagnosis, especially in patients without typical clinical presentation and only high-normal laboratory values [4][56]. Biomarkers mentioned in subsection 1.3.2 like the sFlt1/PIGF ratio oder pp 13 could be used as predictors of PE or HELLP syndrome in early pregnancy [68][1][53]. Control group should be established without cortisone exposure, hence no patients who received lung maturation course. Estimating when delivery will occur and hence timing the administration of prednisolone accordingly has been also found to be beneficial [67]. More research must be conducted in this area, though. It is crucial to stick with the cortisone regimen, even with complicated and early onset cases to prevent the development of a MIX group. A mixture of administrated cortisone derivatives should be avoided as it could lead to over-dose. Partial and incomplete HELLP syndrome has to be considered as different from complete HELLP syndrome and should be treated seperately [43]. Patients with additional autoimmune diseases or refractory cases need to be put in a separate high risk group. They might need closer monitoring and more aggressive treatment. If they turn out to be therapy-refractory, plasma exchange can be an option [43].

In table 5.1 a possible study design is displayed, taking into account everything we learned from this study. This prospective study would focus on a thouroughly conducted diagnosis regimen, close monitoring of clinical features, timed lab draws and cortisone administration.

| Aspects | Details | Time scale |
|---|---|---|
| Diagnosis | ↓ platelets ≤ 100000/$\mu$ l | Day of admission |
| | ↑ liver enzymes ≥ 70 IU/l | |
| | ↓ serum haptoglobine ≤ 41 mg/dl | |
| Clinical examination | Blood pressure, fluid balance | Three times a day |
| Physical examination | Check for signs of RUQP, nausea, vomiting and sight disorder | Once or twice a day |
| Lab variables | HELLP values | Every 24h at the same time |
| | Platelet count, ALT/AST, | Before prednisolone, |
| | Haptoglobin, sFlt1/PIGF ratio | 6h and 12h after |
| | Check coagulation status | Every 24h |
| Prednisolone | Prednisolone 0.5 to 1g | Upon diagnosis then every 12h |
| | Up to three times | |
| Magnesium sulfate | Initially 6 g iv. over 20-30 min | Upon diagnosis max 48h |
| | Maintenance dose 2-3 g/h iv. | |
| Hypertension treatment | iv. Uradipil initially 6.25 mg iv. | Until goal BP ≤140/90 mmHg |
| | maintanance dose 3-24 mg/h [15] | is reached |
| | Oral Nifedipin 5 mg [15] | |

RUQP = Right upper quadrant pain

Table 5.1: Regimen for prospective study

## 5.3 Our concept for HELLP patients

Many centers have performed prospective studies while developing classifications and exercising standarized glucocorticoid regimen [39][38][42][49][57][55]. In the first seven years after introducing our concept into clinical practice we did exactly that except that we used prednisolone as corticoid of choice[65]. We used standardized laboratory values and clinical signs for diagnosis and exerted the our therapeutical concept with the three main pillars: antihypertensive therapy, high-dose prednisolone and magnesium administration. We had some difficulties with the study, as discussed in section 4.9. But we found tendencies suggesting that women with HELLP benefit from our high-dose prednisolone regimen as well as iv. magnesium sulfate therapy, see in subsection 4.8 and 4.6. As current literature shows managment and even diagnosis vary significantly in different

centers. There is a great need for a standardized managment concept for HELLP patients. This is not only important in terms of comparability but for the sake of best possible treatment and consequently outcome for HELLP pregnancies [6]. In figure 5.1 our concept that was administered to PRED patients is illustrated. It shows the different approaches for pre-term and term pregnancies complicated by HELLP syndrome.

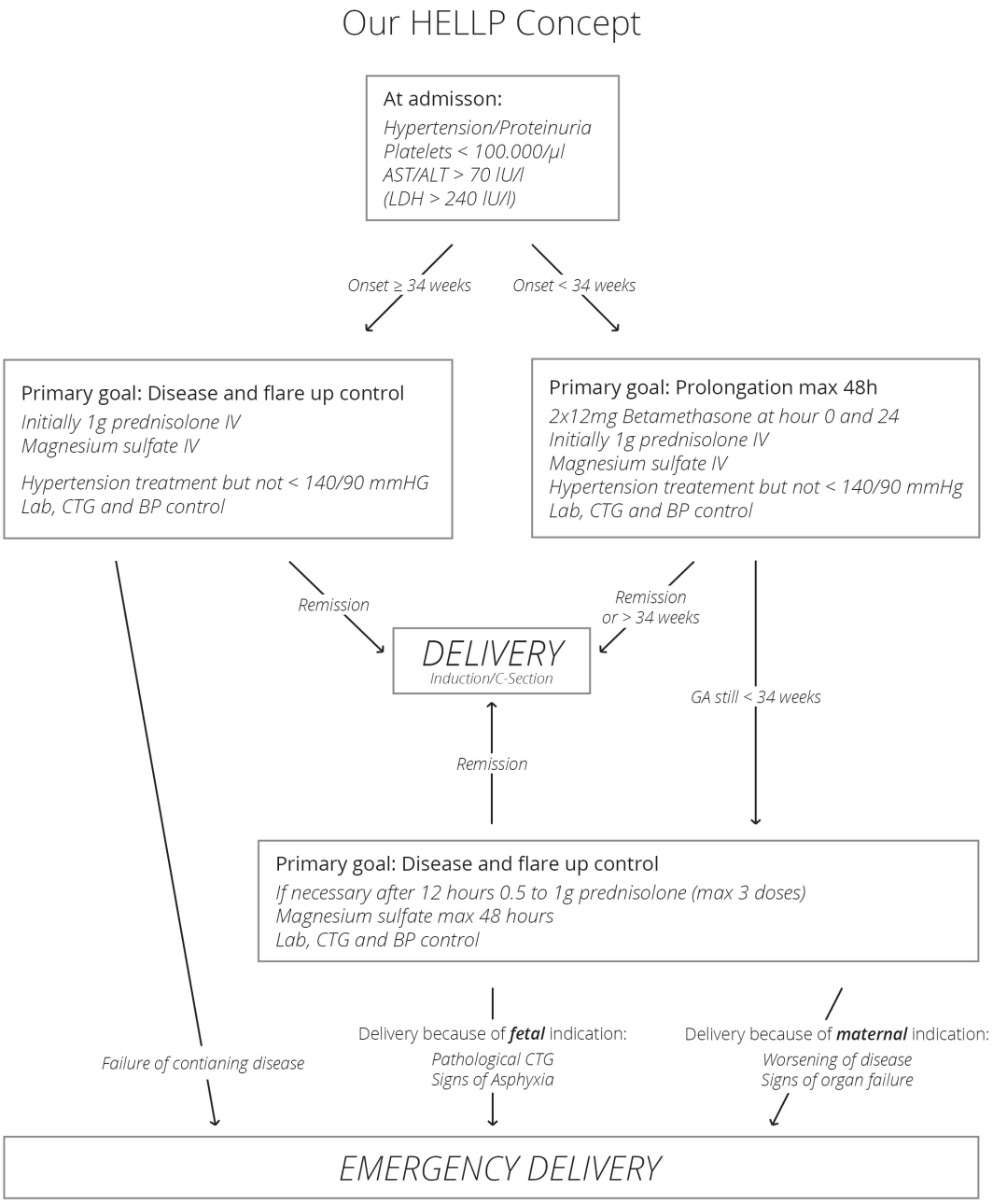


Figure 5.1: Draft of HELLP therapy concept

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