Novel biomarkers in perinatology and infancy

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With the accomplishment of this PhD thesis a dream has come true. In the course of my medical studies in Graz I had already set the goal to perform my PhD studies in clinical research, which in my point of view is the optimal combination of research and clinical work, where you can identify research questions and need for improvement in terms of diagnostic or therapeutic approaches.

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Abstract

Neurofilament light chain (NfL) is a highly specific biomarker reflecting neuroaxonal damage in a variety of acute and degenerative diseases of the brain. It serves in monitoring disease progression and reflecting efficacy of experimental therapies. With measurements solely available in cerebrospinal fluid (CSF) samples at first, novel analytical methods now made it possible to detect NfL in blood samples, which makes it an easily accessible biomarker that reduces the effort of the invasive and laborious procedure of lumbar puncture and enables serial measurements frequently required in disease monitoring.

This PhD thesis aims to apply NfL in a clinical context other than the main field of interest of neurodegenerative disorders, compare it with other existing biomarkers and to test its sensitivity in order to test its applicability in perinatology and infancy. First, we investigated different biomarkers reflecting neuroaxonal damage, stress and hypertension in a cohort of pregnant women at risk of developing preeclampsia, which is a hypertensive disorder of pregnancy and can affect a variety of organ systems including the maternal brain. With preeclampsia being a leading cause of maternal mortality and morbidity there is great need to recognize and treat this feared disorder timely to prevent long-term sequelae. Second, we compared biomarker levels in the same cohort of pregnant women at risk of developing preeclampsia before and after birth since parturition is known to trigger multisystem changes in maternal physiology. Finally, we examined biomarker levels with special attention to NfL in children presenting at the emergency ward with febrile seizures and compared them in children with epileptic seizures and febrile infections without seizures to verify the benign nature of febrile seizures in infancy.

Zusammenfassung

Neurofilament light chain (NfL) ist ein hochspezifischer Biomarker, der neuroaxonalen Schaden in einer Vielzahl von akuten und degenerativen Erkrankungen des Gehirns widerspiegelt. Es hilft dabei, den Krankheitsverlauf zu monitorisieren und gibt Aufschluss über die Wirksamkeit von experimentellen Behandlungen. Zunächst nur in Liquorproben messbar, haben neuartige analytische Methoden den Nachweis von NfL in Blutproben ermöglicht, dies bietet nun einen einfachen Zugang und kann damit die aufwändige, mühsame und invasive Prozedur einer Lumbalpunktion teilweise ersetzen. Zudem vereinfacht es die im Krankheitsverlauf oft notwendigen seriellen Messungen.

Diese PhD-Arbeit zielt darauf ab, NfL in einem anderen klinischen Kontext als das Haupteinsatzgebiet der neurodegenerativen Erkrankungen anzuwenden, mit anderen vorhandenen Biomarkern zu vergleichen und dessen Sensitivität im Einsatzbereich der Perinatologie und im Kindesalter zu prüfen.

Zunächst haben wir verschiedene Biomarker in einer Kohorte von schwangeren Frauen, die das Risiko haben, eine Präeklampsie - eine hypertensive Krankheit der Schwangerschaft, die eine Vielzahl von Organsystemen inklusive des maternalen Gehirns betreffen kann - zu entwicklen, untersucht. Die Biomarker werden häufig eingesetzt, um eine Aussage über neuroaxonalen Schaden, Stress und Hypertension zu tätigen. Da die Präeklampsie eine führende Ursache der maternalen Morbidität und Mortalität ist, gibt es grossen Bedarf, diese Erkrankung frühzeitig zu erkennen und zu behandeln, um Langzeitfolgen zu verhindern. Weiters haben wir Biomarker in derselben Kohorte von schwangeren Frauen vor und nach der Geburt gemessen, da die Geburt per se dafür bekannt ist, Multisystem-Veränderungen in der mütterlichen Physiologie herbeizuführen. Schliesslich haben wir Biomarker in einer Gruppe von Kindern, die sich mit einem Fieberkrampf auf der Notfallstation vorgestellt haben, und in zwei Vergleichsgruppen gemessen, um zu evaluieren, ob es im Rahmen eines Fieberkrampfes zu einem Zerfall von Nervenzellen und damit zu einem Anstieg von NfL kommt. Hiermit konnten wir aufzeigen, dass NfL auch in anderen Fachgebieten zum Einsatz kommen kann und als zuverlässiger Biomarker Auskunft über eine neuronale Beteiligung geben kann.

Abbreviations

ALS	Amyotrophic lateral sclerosis
AVP	Arginine vasopressin
BBB	Blood-brain barrier
BCB	Blood-CSF barrier
BNP	Brain-type natriuretic peptide
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT-proAVP	Copeptin, C-terminal pro-arginine vasopressin
FS	Febrile seizures
IL-1RA	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
MR-proANP	Midregional sequence of pro-A-type natriuretic peptide
MRI	Magnetic resonance imaging
Nf	Neurofilament
NfH	Neurofilament heavy chain
NfL	Neurofilament light chain
NfM	Neurofilament intermediate chain
NSE	Neuron-specific enolase
NT-proBNP	N-terminal fragment of BNP
PE	Preeclampsia
PlGF	Placental growth factor
S100B	S100 calcium-binding protein B
sFlt-1	Soluble fms-like tyrosine kinase-1
Simoa	Single molecule array
VEGF	Vascular endothelial growth factor
VWF	Von Willebrand factor

Introduction

Biomarkers

Biomarkers, a term commonly used in clinical practice and research, is the short form of “biological markers”. These are biological molecules, which can be measured to indicate the state of a biological process from outside the affected individual. The interest in biomarkers has grown sharply in the past decades, which can be visualized in the number of results solely based on the search term “biomarker” on PubMed, whereas the amount of publications has increased tenfold from 1990 with 727 released articles compared with 8800 in 2020. This is not only due to the fact that the possibilities in molecular biology and laboratory testing are constantly improving but also that biomarkers offer simplified options for serial measurements and, of course - if proven to be clinically useful - make economic sense (Agata et al., 1995). Especially in the pediatric population, where physicians strive to follow the principle to perform diagnostics at a minimum to avoid traumatic interventions for the child, the use of biomarkers can support the target of non- or less invasive procedures. Physicians generally aim to initiate and adapt treatment individually, optimally before onset of symptoms in the interest of maintaining health and delaying the advent of disease.

So what are biomarkers? Biomarkers should in general be easily accessible, reflect a biological response of the organism and have physiological ranges whereas values outside of these limit values indicate a condition or a trend of developing this condition. Biomarkers can be obtained from every biological material such as blood, cerebrospinal fluid (CSF), urine, stool, biopsate etc.

In the following chapters the biomarkers, which were evaluated in the framework of the studies and serve as the basis of this PhD thesis, are highlighted briefly.

Neurofilaments

Neurofilaments (Nf) are highly specific major scaffolding proteins of neurons which consist of four subunits: Nf light chain (NfL), Nf medium chain (NfM) and Nf heavy chain (NfH) and depending on the location either α-internexin in the central nervous system (CNS) or peripherin in the peripheral nervous system (Teunissen & Khalil, 2012). These intermediate filaments are important for dendritic branching, growth and stability of axons (Gentil, Tibshirani, & Durham, 2015). In case of axonal damage NfL

is released into the extracellular space and can then be measured in the CSF or blood in the event of axonal damage due to neurodegenerative diseases such as multiple sclerosis and amyotrophic lateral sclerosis (ALS) but also in acute structural cerebral damage in the context of traumatic brain injury (Karantali et al., 2021; Kuhle et al., 2017). In the past decade a substantial breakthrough in the characterization of NfL as a biomarker for various neurodegenerative diseases has taken place (Verde, Otto, & Silani, 2021). Initially only being accessible in the CSF, modern advances have led to the development of a highly sensitive single molecule array (Simoa) immunoassay with the possibility to measure NfL in blood (Disanto et al., 2017). A recent study showed that ratios of blood/CSF NfL levels lie around 1/30 – 1/70 which is comparable with findings in the mouse model (Bacioglu et al., 2016). Various authors have investigated these blood/CSF correlations and could prove that measurements in the blood precisely mirror the events in the CNS. Yet, it is not entirely clear which role the permeability of the blood-brain barrier (BBB) and blood-CSF barrier (BCB) play in the extent of NfL in the blood (Barro, Chitnis, & Weiner, 2020). However, NfL is used as a promising biomarker for disease progression in different nervous system disorders (Lee, Lee, Yip, Chou, & Yip, 2020).

Copeptin

Arginine vasopressin (AVP) also known as antidiuretic hormone is an endogenous hormone, which plays an important role in various basic physiological processes of the organism. Apart from the participation in the homoeostasis of fluid balance it also has great influence on the vascular tonus and the regulation of the endocrine stress response. Since AVP has a short plasma half-life of around 5-15 minutes, direct measurement of AVP is limited and cannot be used routinely (Sklar & Schrier, 1983). Therefore copeptin was found to be a stable and sensitive surrogate marker for AVP measured with a sandwich immunoassay not only for the diagnosis of AVP-dependent fluid disorders (Christ-Crain & Fenske, 2016; Morgenthaler, Struck, Alonso, & Bergmann, 2006). Copeptin derives from the same precursor peptin pre-provasopressin and is the C-terminal portion of provasopressin (de Bree & Burbach, 1998). The readily accessible marker is now an established alternative for detecting nephrogenic diabetes insipidus and is a part of clinical routine diagnostics in many hospitals. Apart from the field of endocrinology copeptin is increasingly used in other areas of expertise such as cardiology, neonatology, obstetrics or intensive care (Balling & Gustafsson, 2014; Evers & Wellmann, 2016; Gaheen, El Amrousy, Hodeib,

& Elnemr, 2021; Henrique et al., 2021; Mieszczański, Górniewski, Błaszczyk, Pacholczyk, & Trzebicki, 2021; Schill, Timpka, Nilsson, Melander, & Enhörning, 2021).

Preeclampsia

Preeclampsia (PE) is a hypertensive multisystem disorder during pregnancy and is discussed to emerge on the one hand due to maternal cardiovascular performance resulting in uteroplacental hypoperfusion and on the other hand to be a primary placental disorder. Worldwide PE is related to 2% to 8% pregnancy-related complications (Karrar & Hong, 2021). Soluble factors are released into the circulation which lead to hypertension and multi-organ injury following vascular endothelial injury (L. C. Chappell, Cluver, Kingdom, & Tong, 2021). PE is characterized by the new onset of hypertension of either proteinuria or a significant end-organ dysfunction with or without proteinuria with symptoms beginning after 20 weeks into pregnancy, with an onset mostly after 34 weeks (Phipps, Prasanna, Brima, & Jim, 2016). Preeclampsia is a major cause of maternal and perinatal mortality and morbidity with over 70 000 maternal deaths and 500 000 fetal deaths worldwide every year and has no known curative treatment with delivery of the child and the placenta being the only cure whereby symptoms can also persist after birth (Lucy C. Chappell, Cluver, Kingdom, & Tong; Powles & Gandhi, 2017; Rana, Lemoine, Granger, & Karumanchi, 2019). Various maternal characteristics have been found to be risk factors for the development of PE, among others parity, a history of PE in an earlier pregnancy, chronic hypertension and maternal age play an important role in the development of the disorder (Anderson, Olsson, Kristensen, Åkerström, & Hansson, 2012; Rana et al., 2019).

sFlt-1/PlGF ratio

Soluble fms-like tyrosine kinase-1 (sFlt-1) is an antiangiogenic agent made by the placenta and is upregulated in preeclamptic patients whereas placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) are proangiogenic factors which can be downregulated by hypoxia (Khaliq et al., 1999). The balance among sFlt-1 and PlGF is important for normal placental development; if this is not present, systemic endothelial dysfunction can occur (Herraiz, Llurba, Verlohren, & Galindo, 2018; Lecarpentier & Tsatsaris, 2016). Several studies have been performed on serum concentrations of angiogenic factors in pregnant women (Binder, Kalafat, Palmrich, Pateisky, & Khalil, 2021; Jeon et al., 2021; Levine et al., 2004; Lim et al.,

2021), measurement of the ratio of sFlt-1 to PlGF seems to be a convenient test to exclude PE in women with suspected PE, with a cut-off of ≤ 38 having a negative predictive value of 99.3% for developing PE in the subsequent week (Zeisler et al., 2016). Since hypertensive disorders during pregnancy can have significant clinical impact also on the child´s health such as congenital heart defects, higher blood pressure during childhood and adverse cognitive outcomes, there is great need for prompt diagnosis, adequate treatment and timely management, so multi-marker models for prediction of PE are of special interest for the physicians in charge (Duley, Meher, & Abalos, 2006; Kanata, Liazou, Chainoglou, Kotsis, & Stabouli, 2021; Sibai & Barton, 2007).

Peripartum-induced brain changes

Peripartum-associated changes affect several systems of the maternal body during pregnancy, birth und lactation. Although a lot of researchers have dedicated themselves to the topic around parturition, many processes cannot be explained yet. Apart from major alterations to the endocrine system pregnancy also leads to changes in the cardiovascular system due to a rise in blood volume and entails dynamic structural and functional changes of the maternal brain and thus has great impact on behavior in order to adapt to the child's needs during motherhood (Barba-Müller, Craddock, Carmona, & Hoekzema, 2019). The modifications that take place range from neurogenesis, synaptic remodeling and changes in dendritic morphometry (Kinsley et al., 2006; Leuner & Sabihi, 2016). Few studies have focused on neuroimaging in aiming on visual representation of structural brain changes (Hillerer, Jacobs, Fischer, & Aigner, 2014; Pilyoung Kim, 2016; P. Kim, Strathearn, & Swain, 2016). Almost two decades ago Oatridge et al. were able to show reversible reduction in brain size in pregnant women (Oatridge et al., 2002). Recently it was shown that first-time mothers undergo extensive gray matter volume reductions across pregnancy lasting for at least 2 years postpartum with a statement of the authors that these changes predicted measures of postpartum maternal attachment (Hoekzema et al., 2017). Current evidence shows that the volume reduction persists six years after parturition which opens the possibility that pregnancy-induced brain changes are permanent (Martínez-García et al., 2021). This is supported by the findings of the Rotterdam Study where long-term changes in the brain structure involving larger global gray matter volume that persists for decades are discussed (Aleknaviciute et al., 2021). In addition to this, the group around de

Lange et al. demonstrated that parous women showed less evidence of brain aging compared to their nulliparous peers (de Lange et al., 2019). To date studies involving changes in neuronal biomarkers during pregnancy and parturition are scarce.

Febrile seizures

Febrile seizures (FS) are seizures, which occur in children from six months to five years of age and are accompanied by fever without central nervous infection, hypoglycemia or electrolyte imbalance. They can either be simple of complex, whereas the latter are associated with focal neurologic findings, last for more than fifteen minutes and are recurrent within 24 hours. FS arise from a vulnerability of the developing central nervous system to the effects of fever; furthermore the affected children have an underlying genetic predisposition and are influenced by environmental factors (Leung, Hon, & Leung, 2018). Since the condition is benign and self-limiting most children have a very good prognosis after presenting FS, nevertheless the events are stressful and frightening for the caregivers (Laino, Mencaroni, & Esposito, 2018; Leung et al., 2018; Smith, Sadler, & Benedum, 2019). To date the diagnosis of FS is clinical due to the lack of objective postictal biomarkers. Recently published studies have shown that circulating copeptin has high diagnostic accuracy in FS, that FS are associated with higher levels of von Willebrand factor (VWF) parameters and especially VWF:collagen binding activity may serve as additional biomarker in the diagnosis of FS (Pechmann, Wellmann, Stoecklin, Krüger, & Zieger, 2019; Stöcklin et al., 2015). Apart from these findings around two decades ago, a Japanese group evaluated whether FS caused brain damage by measuring the marker Neuron-specific enolase (NSE) in 53 patients and showed that FS seldom cause severe neurologic damage (Tanabe et al., 2001). Prior to our study Neurofilaments had only been investigated in prolonged febrile seizures, the results suggested that prolonged febrile seizures could lead to some degree of neuronal damage (Matsushige et al., 2012).

Aim of the present work and hypothesis

The risk for sentinel events is highest around birth. At the end of pregnancy, during birth and the first days afterwards, various perinatal complications may pose great danger as well to the mother as to the child. Unfortunately, individual clinical prediction of preeclampsia and preeclampsia-related complications is hardly possible

even though it is a major area of current research. Thus, in perinatology there is a need to evaluate promising novel biomarkers for their potential to support clinical assessment in this vulnerable population. Additionally and in order to reduce fetal and perinatal morbidity and mortality, improved monitoring of potentially dangerous maternal conditions during pregnancy is needed.

In both perinatology and pregnancy novel fluid biomarkers constitute a very elegant method to predict medical conditions that need early intervention. The advantages compared to other early detection methods are diverse: there is no radiation exposure, biomarkers can be easily detected in samples of urine, CSF or peripheral blood and the costs are reasonable. In this PhD thesis different novel biomarkers were investigated, namely PlGF, sFlt-1, the axonal injury marker NfL and the neuroendocrine stress marker copeptin. All biomarkers were measured in serum samples of pregnant women in a prospective observational study during pregnancy.

Together, we hypothesized, that by combining information of novel serum biomarkers with established biomarkers and clinical parameters in a computer model in pregnant women, early diagnosis and prediction of preeclampsia can be improved which would empower obstetricians to do a better risk stratification and to plan adequate therapy more precisely. Second, neurological problems resemble a key criteria in preeclampsia but it is unknown if this is paralleled by axonal loss. By measuring serum Nf we addressed this issue.

Furthermore we set our focus on the investigation of the impact of parturition on maternal cardiovascular and neuronal integrity by comparison of pre- versus post-delivery maternal serum biomarkers since pregnancy and birth seem to lead to profound multisystem changes and cause significant changes in a woman’s brain.

Finally we investigated the impact of febrile and epileptic seizures on serum NfL, serum copeptin and prolactin levels in children aging six months to five years. We compared the biomarker levels with those of children with febrile infections without convulsions in order to evaluate if the benign nature of febrile seizures can be related to corresponding biomarker levels.

Publications

Manuscript 1: Neurofilament as Neuronal Injury Blood Marker in Preeclampsia

Journal: Hypertension - published

Authors: Katrina Suzanne Evers,* Andrew Atkinson,* Christian Barro, Urs Fisch, Marc Pfister, Evelyn A. Huhn, Olav Lapaire, Jens Kuhle, Sven Wellmann

Abstract:

Preeclampsia has been shown to be associated with changes in cerebral structure and cognitive function later in life. Nf (neurofilaments) are specific scaffolding proteins of neurons, and their quantification in serum has been proposed as a biomarker for neuroaxonal injury. We performed a prospective, longitudinal, single-center study at the University Hospital of Basel to determine serum Nf concentrations in pregnant women with singleton pregnancies and with high risk of preeclampsia or with early signs of preeclampsia. Enrollment started at 21 weeks of gestation, followed up with multiple visits until delivery. Sixty out of 197 women developed preeclampsia (30.5%). NfL (Nf light chain) was measured with a highly sensitive single molecule array (Simoa) assay, in addition to the established preeclampsia markers sFlt-1 (soluble fms-like tyrosine kinase-1) and PlGF (placental growth factor). The most important independent predictors of NfL were maternal age, number of pregnancies, and proteinuria. NfL levels increased during pregnancy and were significantly higher in women developing preeclampsia. The discriminatory accuracy of NfL, PlGF, and sFlt-1 in receiver operating characteristic curves analysis (area under the curve) of the overall group was 0.68, 0.81, and 0.84, respectively, and in women older than 36 years 0.7, 0.62, and 0.79, respectively. We conclude that increased axonal injury serum marker NfL predicts preeclampsia particularly in older women, with an accuracy similar to the established angiogenic factors. NfL may serve as an early indicator of preeclampsia-induced changes in cerebral structure and may help to stratify disease management.

Neurofilament as Neuronal Injury Blood Marker in Preeclampsia

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Abstract —Preeclampsia has been shown to be associated with changes in cerebral structure and cognitive function later in life. Nf (neurofilaments) are specific scaffolding proteins of neurons, and their quantification in serum has been proposed as a biomarker for neuroaxonal injury. We performed a prospective, longitudinal, single-center study at the University Hospital of Basel to determine serum Nf concentrations in pregnant women with singleton pregnancies and with high risk of preeclampsia or with early signs of preeclampsia. Enrollment started at 21 weeks of gestation, followed up with multiple visits until delivery. Sixty out of 197 women developed preeclampsia (30.5%). NfL (Nf light chain) was measured with a highly sensitive single molecule array (Simoa) assay, in addition to the established preeclampsia markers sFlt-1 (soluble fms-like tyrosine kinase-1) and PlGF (placental growth factor). The most important independent predictors of NfL were maternal age, number of pregnancies, and proteinuria. NfL levels increased during pregnancy and were significantly higher in women developing preeclampsia. The discriminatory accuracy of NfL, PlGF, and sFlt-1 in receiver operating characteristic curves analysis (area under the curve) of the overall group was 0.68, 0.81, and 0.84, respectively, and in women older than 36 years 0.7, 0.62, and 0.79, respectively. We conclude that increased axonal injury serum marker NfL predicts preeclampsia particularly in older women, with an accuracy similar to the established angiogenic factors. NfL may serve as an early indicator of preeclampsia-induced changes in cerebral structure and may help to stratify disease management. **(*Hypertension*. 2018;71:1178-1184. DOI: 10.1161/HYPERTENSIONAHA.117.10314.)** ● **Online Data Supplement**

Key Words: biomarker ■ blood-brain barrier ■ hypertension ■ preeclampsia ■ pregnancy

Worldwide hypertensive disorders of pregnancy are a serious health issue for women and their children, accounting for 12% of maternal deaths. In high-income countries, it affects between 2% and 8% of all pregnancies.[1] The most important hypertensive disorder in pregnancy is preeclampsia.[2] Preeclampsia is a pregnancy-specific syndrome defined by the new onset of hypertension after 20 weeks of gestation and by proteinuria.[2,3] Additional symptoms frequently complicate preeclampsia such as neurological symptoms, epigastric pain with nausea or vomiting, thrombocytopenia, and abnormal liver enzymes. This complication of preeclampsia is known as HELLP syndrome, with H standing for hemolysis, EL for elevated liver enzyme levels, and LP for low platelet levels.[4] Pregnant women have an increased risk of developing preeclampsia if there is, for example, and just to mention the most common, a previous history of preeclampsia, multiple pregnancies, preexisting diabetes mellitus, nulliparity, high body mass index before pregnancy, or maternal age ≥40 years.[5]

Over the last decade, a variety of translational biomarker studies improved fundamentally our understanding and diagnosis of preeclampsia mainly addressing the cardiovascular dysfunction of the disease.[6,7] Preeclampsia is a heterogeneous, multi-system disorder and can impair the central nervous system (CNS).[8] Certain neurological symptoms may precede the onset of seizures, such as persistent headaches, blurred vision, photophobia, and altered mental status.[9] Furthermore, preeclampsia may cause long-term cognitive changes and increased lifetime risk of cerebrovascular stroke.[10] Preeclampsia is associated with long-term cerebral white and gray matter changes and cognitive impairment.[11,12]

Nf (neurofilaments) are highly specific major scaffolding proteins of neurons consisting of 4 subunits: the triplet of NfL (Nf light), Nf medium, and NfH (Nf heavy) chains and α-internexin in the CNS, or peripherin in the peripheral nervous system.[13] Disruption of the axonal cell membrane because of acute or chronic neuronal damage releases Nf into

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the interstitial fluid and eventually to the cerebrospinal fluid and the blood compartment.[14,15] Significantly increased serum concentrations of Nf were found after acute brain injury such as ischemic or hemorrhagic stroke, traumatic brain injury, cervical artery dissection, and spinal cord injury compared with healthy individuals,[16–19] and also in patients with multiple sclerosis and various neurodegenerative diseases.[20,21] Recent advances using highly sensitive Simoa technology improved the detection of NfL particularly in peripheral blood making NfL a promising and an easily accessible biomarker for neuroaxonal injury.[22–25]

The aim of this study was to (1) measure serum NfL concentrations at multiple visits in a cohort of pregnant women with high risk of developing preeclampsia or with early signs of preeclampsia, (2) investigate clinical factors that influence serum NfL concentrations, and (3) study the prognostic value of NfL compared with the established angiogenic factors sFlt-1 (soluble fms-like tyrosine kinase-1) and PlGF (placental growth factor) in predicting preeclampsia.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

This prospective, observational study was conducted at the University Hospital of Basel between 2011 and 2015. The Ethics Committee of Northwestern Switzerland (PB_2016-02490) approved the study protocol, and written informed consent was obtained from all participating women.

Women >18 years of age with a singleton pregnancy were included if they presented with at least 1 risk factor for preeclampsia: nulliparous obese women with body mass index >26.1 kg/m^2, nulliparous women >40 years of age, preexisting diabetes mellitus, essential hypertension or renal disease, pregnancy-induced hypertension, gestational diabetes mellitus, uteroplacental dysfunction, previous preeclampsia, eclampsia or HELLP, thrombophilia, antiphospholipid antibodies, family history of preeclampsia, or eclampsia or HELLP in first-line relatives. Women with hypertension (systolic blood pressure [BP] >140 and diastolic BP >90 mm Hg) and proteinuria (>1+ in dipstick which corresponds to a protein concentration ≥30 mg/dL protein in spot urine) were also included as these clinical findings are associated with increased risk of preeclampsia. Exclusion criteria were chromosomal aberrations, fetal malformations, abortion, or stillbirth <22 weeks of gestation. All eligible women were enrolled into the study during antenatal visits starting with first visit at 21 to 24 weeks of gestation (visit 0) or during hospitalization at 21 weeks of gestation or later. Thus, a rolling enrollment was allowed. Women were followed up every 2 to 4 weeks with study visits including recording of demographic characteristics, medical history, clinical examinations, and drawing blood for biomarker analyses (sFlt-1, PlGF, and NfL). The study comprised up to 5 visits, visit 0 at 21 to 24 weeks, visit 1 at 25 to 29, visit 2 at 29 to 32, visit 3 at 33 to 36, and visit 4 at 37 to 40 weeks of gestation. Women with symptoms of preeclampsia and those at increased risk of preeclampsia and suggestive clinical findings were controlled clinically and biochemically from minimum every day to maximum every 14 days dependent on their clinical condition until delivery. Results of biomarker analyses were not available until the end of study, hence did not influence clinical management and treatment.

Diagnostic Criteria for Hypertension-Associated Diseases in Pregnancy

Diagnostic criteria for preeclampsia were new-onset systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg measured on 2 occasions at least 4 hours apart but within 1 week, and new onset of proteinuria with ≥300 mg/24 h urine protein collection or ≥2+ in dipstick >20 weeks of gestation.[2] Diagnostic criteria for each preeclampsia-related disorder were based on international guidelines (Table S1 in the online-only Data Supplement).[2] Neurological symptoms included headache, confusion, and visual disturbances.[26]

Sample Preparation and Assessment of PlGF, sFlt-1, and NfL

Serum samples were collected and processed according to a standard operating procedure, defining venipuncture of the antecubital vein, subsequent sample transfer to the central laboratory service, centrifugation, preparation of aliquots, and storage at −80°C until batch-wise analysis. All samples used in the current analysis had not previously been thawed. Researchers performing the assays were blinded to the patients' clinical information and pregnancy outcome.

Maternal serum levels of sFlt-1 and PlGF were measured using Roche Elecsys assays on the electrochemiluminescence immunoassay platforms, Modular E170 (Roche Diagnostics) until October 2014 and Cobas 6000 (Roche Diagnostics) from November 2014 until the end of the study. The within-run coefficient of variation for quality control samples was <1.5% for the sFlt-1 and <0.9% for the PlGF assay on Modular E170. Between-run coefficients of variation are 2.5% to 3.9% for the sFlt-1 and 2.7% to 3.7% for the PlGF assay on Modular E170 and 1.2% to 2.3% for the sFlt-1 and 1.7% to 2.0% for the PlGF assay on Cobas 6000.

The concentration of NfL was determined using a Simoa assay, which was established using the NF-light assay ELISA kit from UmanDiagnostics (Umeå, Sweden), transferred onto the Simoa platform with a homebrew kit (Quanterix Corp, Boston, MA), and has been described in detail by our group elsewhere.[25] Calibrators (neat) and serum samples (1:4 dilution) were measured in duplicates. Bovine lyophilized NfL was obtained from UmanDiagnostics. Calibrators ranged from 0 to 2000 pg/mL. Batch prepared calibrators were stored at −80°C. Intra- and interassay variabilities of the assay were <10%. Repeated measuring was performed for the few samples with intra-assay coefficients of variation >20%.

Statistical Analysis

In terms of descriptive statistics, tests for difference between patients with and without preeclampsia were performed using the Student *t* test for normally distributed continuous variables, with the Mann–Whitney–Wilcoxon test for non-normally distributed continuous variables, or with Pearson χ^2 test for dichotomous variables.

Univariate and multivariate-adjusted logistic models were fitted with final preeclampsia diagnosis as dependent variable with baseline (visit 3) categorical variables, ethnicity (4 levels), number of pregnancies (8 levels), parity (5 levels), smoking status (3 levels), neurological symptoms (yes/no) and proteinuria (yes/no) and covariates age, body mass index, systolic BP, PlGF, and sFlt-1 as independent variables.

The predictive power of the model was validated using cross-validation. Forest plots were used to compare the area under the curve (AUC) from the resulting plots. receiver operating characteristic curves shown are from univariate models.[27]

A post hoc subgroup analysis was performed by dividing patients by age according to a specific cutoff point; results are shown for the groups based on the median age (36 years, groups being 18–36 and 37–49).

To investigate NfL effects in more detail, an adjusted linear mixed-effects model was fitted with NfL as dependent variable and the above-mentioned variables as fixed-effect regressors with random intercept and slope effects for the visit.

All analyses were conducted with the statistical package R (R Foundation for Statistical Computing), and a value <0.05 was considered statistically significant. Further information is provided in the online-only Data Supplement.

Results

Between September 1, 2011, and June 30, 2015, a total of 236 women between the ages of 18 to 49 years were recruited for the study. Thirty-nine women dropped out because of

nonavailability of serum samples for this study. Finally, a total of 197 women were included, 85% were White, 10% Asian, 2% Black, and 3% other or missing. Sixty women developed preeclampsia (30.5%) of whom 30 women (50%) developed early-onset preeclampsia (before 34 weeks of gestation). The triplet of serum biomarkers PlGF, sFlt-1, and NfL was measured in 410 serum samples of these 197 women; thus, on average, there were 2 visits per women. Table 1 summarizes the baseline characteristics with *P* values for the comparison of patients with and without preeclampsia during the study.

In the mixed-effects linear model with NfL as a dependent variable, preeclampsia status ($P<0.0001$), maternal age ($P<0.001$), number of pregnancies ($P<0.001$), and proteinuria ($P<0.001$) had a significant and independent influence on NfL, whereas, unexpectedly, systolic BP and neurological symptoms were not identified as significant factors influencing serum NfL concentrations (Tables S3).

NfL levels were higher in women with preeclampsia when compared with women not developing preeclampsia (controls; Figure 1, upper; Table S2). NfL increased with increasing maternal age with a steeper increase in women with preeclampsia compared with controls (Figure 1, lower).

In univariate logistic regression analyses, increased levels of NfL and sFlt-1 were associated with an increased risk of preeclampsia, whereas increased PlGF levels showed a reduced risk for preeclampsia (Table 2 with preeclampsia as dependent variable). In addition, criteria that lead to the diagnosis of preeclampsia (systolic BP and proteinuria) and the associated neurological symptoms were also significant factors for increasing the risk of preeclampsia. After adjusting for various factors, NfL remained predictive for preeclampsia (Table 2).

Receiver operating characteristic curve analysis revealed that in the overall group, NfL (AUC, 0.68; 95% confidence interval [CI], 0.60–0.77) was not superior to the established biomarkers sFlt-1 (AUC, 0.84; 95% CI, 0.77–0.91) and PlGF (AUC, 0.81; 95% CI, 0.74, 0.87) to predict preeclampsia (Figure 2, left). The predictive ability of NfL enhanced in the age group >36 years (NfL: AUC, 0.7; 95% CI, 0.5–0.9 versus sFlt-1: AUC, 0.79; 95% CI, 0.61–0.96 versus PlGF: AUC, 0.62; 95% CI, 0.44–0.81; Figure 2, right).

Discussion

NfL is a highly specific structural protein of neurons. Elevated serum levels of NfL are increasingly recognized as measures of acute or chronic neuroaxonal damage. In this prospective longitudinal study, enrolling pregnant women at increased risk to develop preeclampsia or with early signs of preeclampsia after 20 weeks of gestation, we measured NfL levels together with the established preeclampsia markers sFlt-1 and PlGF at multiple visits until delivery. The major findings are (1) women developing preeclampsia have elevated levels of NfL compared with women without preeclampsia, (2) there is a positive correlation of NfL with maternal age, and (3) NfL is of predictive value for preeclampsia especially in women >36 years.

Cerebral autoregulation confers constant cerebral blood flow over a wide range of systemic BP ranging from 60 to 150

Table 1. Baseline and Delivery Characteristics

	PE (n=60)	No PE (n=137)	*P* Value
Baseline characteristics			
Age (y) (median [IQR])	34 [30–36]	33 [29–36]	0.53
BMI (median [IQR])	30 [27–32]	29 [26–34]	0.744
Systolic blood pressure, mm Hg	160 [145–180]	127 [115–135]	<0.001
Any neurological symptoms (%)	26 (43.3)	7 (5.1)	<0.001
Dipstick (≥2+) (%)	37 (61.7)	5 (3.6)	<0.001
Parity (%)			0.279
0	34 (56.7)	78 (56.9)	
≥1	26 (43.3)	59 (43.1)	
Smoker (%)			0.04
Never	43 (71.7)	97 (70.8)	
Past	7 (11.7)	11 (8)	
Current	1 (1.7)	18 (13.1)	
Missing	9 (15)	11 (8)	
Ethnicity (%)			0.241
White	50 (83.3)	117 (85.4)	
Asian	4 (6.7)	16 (11.7)	
Black	2 (3.3)	2 (1.5)	
Other	2 (3.3)	1 (0.7)	
Missing	2 (3.3)	1 (0.7)	
Delivery characteristics			
Delivery mode (%)			0.106
Cesarean section	46 (76.7)	80 (58.4)	
Vaginal	14 (23.3)	57 (41.6)	
Gender (%)			0.507
Girls	32 (54.7)	62 (46.7)	
Boys	28 (45.3)	75 (53.3)	
GA at birth, days (median [IQR])	245 [206–267]	271 [260–277]	<0.001
Cord pH (median [IQR])	7.28 [7.24–7.32]	7.28 [7.23–7.32]	0.96
Birthweight in g (median [IQR])	2040 [1135–2835]	3042.5 [2474–3600]	<0.001
Biomarkers at baseline			
NfL pg/mL (median [IQR])	28.7 [17.9–47.3]	18.2 [13.8–24.6]	<0.001
PlGF pg/mL (median [IQR])	65 [30–110]	256 [118–500]	<0.001
sFlt-1 pg/mL (median [IQR])	10432 [7259–13531]	2714 [1655–4902]	<0.001

Data are expressed as median [IQR] or number of cases per category (%). GA indicates gestational age; IQR, interquartile range; NfL, neurofilaments light chain; PE, preeclampsia; PlGF, placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1.

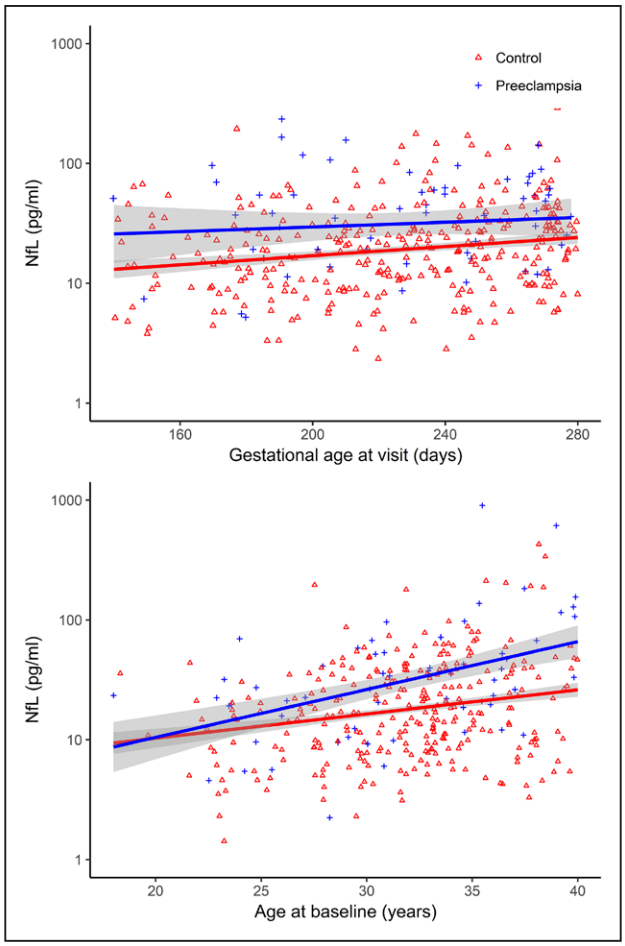

Figure 1. NfL (neurofilaments light chain) level during pregnancy. Log_{10} NfL plotted against gestational age (**upper**) and mother's age (**lower**) at visit stratified by final preeclampsia (PE) status (controls=red triangles, PE=blue crosses), with fitted linear model (solid blue line=PE, red solid line=controls) including 95% confidence intervals (shaded).

Table 2. Univariate and Multivariate Models Testing Associations Between Biomarkers or Clinical Characteristics and Final Diagnosis of Preeclampsia

Characteristics	Univariate Models		Adjusted Final* Model	
	Odds Ratio [95% CI]	*P* Value	Odds Ratio [95% CI]	*P* Value
NfL (10 step)	1.24 [1.09–1.44]	0.002	1.19 [1.06–1.39]	0.02
PlGF (100 step)	0.42 [0.28–0.58]	<0.001	nE	
sFlt-1 (1000 step)	1.35 [1.24–1.49]	<0.001	nE	
Age (per year)	1.01 [0.96–1.07]	NS	nE	
Ethnicity			nE	
White	1 (reference)			
Non-white	0.98 [0.38–2.33]	0.97		
log_{10} BMI	1.61 [0.04–55.4]	0.79	nE	
Gravidity	0.71 [0.52–0.95]	0.02	0.75 [0.50–1.08]	0.15
Parity	0.93 [0.62–1.38]	0.73	nE	
Smoker			nE	
No	1 (reference)			
Yes	0.62 [0.25–1.42]	0.28		
Gestational diabetes mellitus				0.17
No	1 (reference)		1 (reference)	
Yes	0.45 [0.18–0.99]	0.06	0.48 [0.16–1.30]	
Systolic BP ≥140 mm Hg			nE	
No	1 (reference)			
Yes	26.44 [12.23–61.49]	<0.001		
Neurological symptoms				
No	1 (reference)		1 (reference)	
Yes	14.74 [6.14–39.77]	<0.001	15.89 [6.47–43.7]	<0.001
Proteinuria ≥2+ dipstick			nE	
No	1 (reference)			
Yes	50.24 [19.02–60.88]	<0.001		

Odds ratios with 95% confidence intervals (CIs) from fitting univariate (left) and multivariate-adjusted logistic regression models (right) with dependent variable final preeclampsia status. BMI indicates body mass index; nE, not estimated in final model; NfL, neurofilaments light chain; NS, not significant at the 5% level; PlGF, placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1

*Final adjusted model determined via stepwise backwards selection using Akaike and Bayesian information criteria; PlGF and sFlt-1 not considered as they are existing biomarkers; BP (blood pressure) and dipstick not included as these are currently used diagnostics. Only those covariates found to be significant risk factors in the univariate analysis were carried forward to the multivariate analysis.

mm Hg.[28] Outside this range, autoregulation is lost and significant brain tissue damage can occur because of hyperperfusion, blood–brain barrier (BBB) disruption, and vasogenic edema formation.[29] The BBB is the interface between the circulating blood and the brain and functions as gatekeeper for substances between both blood and brain.[30] Women experiencing preeclampsia are at increased risk for hampered autoregulation and subsequent cerebral edema because of severe hypertension.[31–33] In addition, in preeclampsia, a large variety of circulating factors is increased, of which at least some directly impair BBB integrity.[34,35] Brain edema identified by magnetic resonance imaging in patients with preeclampsia was associated with BBB disruption and abnormalities in endothelial damage markers but not with hypertension level.[36]

In our multivariate linear regression analyses performed with NfL as dependent variable, neither systolic BP nor proteinuria were significant predictors of NfL. However, maternal age and sFlt-1 were factors, indicating that distinct changes in this multi-system disorder are stronger predictors of NfL than the clinical hallmarks hypertension and proteinuria.

Few studies investigated different peripheral biomarkers of CNS injury in preeclampsia and reported increased values of the calcium-binding protein S100B and the neuron-specific enolase,[37–40] supporting the notion of possible cerebrovascular dysfunction and BBB disruption in preeclampsia.[41] In our prospective cohort, no women developed severe cerebral complications such as seizures or stroke. Thus, the broad elevation of NfL in women with preeclampsia may indicate subclinical cerebral involvement.

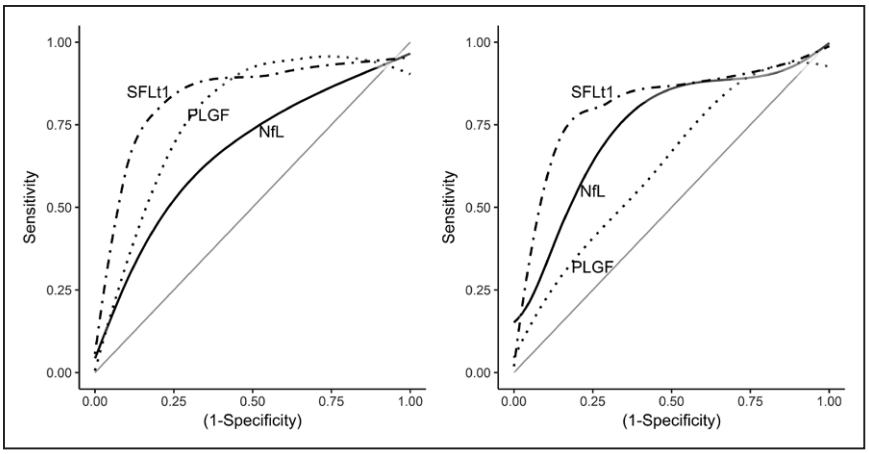

Figure 2. Predictive value of NfL (neurofilaments light chain) for preeclampsia. Comparison of receiver operating characteristic (ROC) curves and area under the curve (AUC) values with forest plots of the overall group (**left**) and of the age group >36 years (**right**). PlGF indicates placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1.

In addition to the longitudinal study design, a further strength of our study is the measurement of sFlt-1 and PlGF in all samples in parallel to NfL. Both sFlt-1 and PlGF have gained great attention in recent years as highly sensitive and specific markers for the assessment of women with suspected preeclampsia.[42] On the basis of the whole cohort, we found high AUC values for predicting preeclampsia of sFlt-1 and PlGF and a lower value for NfL (Figure 2, left). When considering the effect of aging on NfL and dividing the study population into 2 groups based on the median of maternal age, the AUC of NfL improved compared with sFlt-1 and PlGF (Figure 2, right). The higher predictive value of NfL in women >36 years is in line with the findings from the mixed-linear effect model fitted with NfL as dependent variable. Both modeling approaches seem to indicate that NfL increases with aging in women with preeclampsia stronger than in the control group.

An association of NfL with age in healthy controls has been reported consistently,[43–48] whereas it is typically absent in established multiple sclerosis (relapsing-remitting multiple sclerosis,[20,47,49] secondary progressive multiple sclerosis,[50] and primary progressive multiple sclerosis[47]) and in patients with a clinically isolated syndrome.[46,47,49] The finding that Nf levels in serum are in close correlation with those in cerebrospinal fluid marks a breakthrough for its routine use in intraindividual longitudinal assessments of multiple sclerosis and in primary neurodegenerative disorders like amyotrophic lateral sclerosis and frontotemporal dementia.[25,51–57] The high association between these 2 compartments is the prerequisite to support the assumption that blood NfL measurements indeed reflect CNS neuroaxonal injury.[24] With the advent of the single molecule array (Simoa) technology as a digital immunoassay, the sensitivity has significantly improved and allows the reliable and accurate quantification of serum NfL levels in healthy controls.[22,58–60] On the basis of this analytic platform, the high correlation for NfL levels between the cerebrospinal fluid and blood compartment has been confirmed in multiple sclerosis,[25] as well as in other diseases like HIV infection,[60] progressive supranuclear palsy,[61] boxers after bout,[24] mild cognitive impairment/Alzheimer's disease, and cognitively normal controls.[62]

In our study, only pregnant women at increased risk of developing preeclampsia or with early signs of preeclampsia have been enrolled. Thus, conclusions drawn on the controls in this study must be interpreted carefully, especially with respect to healthy pregnancies, and we would recommend additional investigations on NfL in this regard. In fact, there is a lack of published information on NfL levels for healthy women with or without pregnancy. A further limitation of our study is the limited number of longitudinal measurements per women. On average, we collected 2 samples per study participant, and there was a considerable variability in the number of visits attended by the women, as highlighted in Figure S1 which describes the trajectories of NfL values over all 5 visits.

We suggest that further studies investigate these baseline values, stratified by age group, to test our 2 main hypothesis from the study, namely, that age plays a role of the rise of NfL levels, and second, that having preeclampsia may affect BBB and CNS tissue integrity.

Perspectives

Our results show that NfL levels are increased in pregnant women at high risk of preeclampsia or with early signs of preeclampsia in comparison to those who did not and that NfL may have predictive value for preeclampsia particularly in older pregnant women. Our study is expected to motivate further investigations as to examine closely the association of NfL levels with cerebral symptoms and to combine NfL measurements with magnetic resonance imaging investigations. This would potentially pave the way to establish NfL as an early indicator of preeclampsia-induced neuroaxonal injury and to use NfL as an adjunct in preeclampsia management similar to NfL perspectives in neurodegenerative diseases.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Serum levels of NfL (neurofilament light chain) are increased in pregnant women with confirmed preeclampsia, particularly in older women.

What Is Relevant?

- Because NfL is released exclusively from neurons, increased serum levels in pregnant women developing preeclampsia indicate neuronal injury. Therefore, preeclampsia is a brain damaging disease. Future studies may examine the effect of specific neuroprotection, for example, by tight antihypertensive treatment on the release of NfL in pregnant women developing preeclampsia.

Summary

Increased neuronal injury serum marker NfL in pregnant women with preeclampsia points toward preeclampsia-related damage of the nervous system. This finding corroborates very recent reports on persistent structural and functional brain impairment in women after preeclampsia.

Manuscript 2: Impact of parturition on maternal cardiovascular and neuronal integrity in a high risk cohort – a prospective cohort study

Journal: BMC Pregnancy and Childbirth – published

Authors: Katrina Suzanne Evers, Evelyn Annegret Huhn, Sotirios Fouzas, Christian Barro, Jens Kuhle, Urs Fisch, Luca Bernasconi, Olav Lapaire and Sven Wellmann

Abstract:

Background: To better understand the profound multisystem changes in maternal physiology triggered by parturition, in particular in the underexplored neuronal system, by deploying a panel of pre- vs post-delivery maternal serum biomarkers, most notably the neuronal cytoskeleton constituent neurofilament light chain (NfL). This promising fluid biomarker is not only increasingly applied to investigate disease progression in numerous brain diseases, particularly in proteopathies, but also in detection of traumatic brain injury or monitoring neuroaxonal injury after ischemic stroke.

Methods: The study was nested within a prospective cohort study of pregnant women at risk of developing preeclampsia at the University Hospital of Basel. Paired ante- and postpartum levels of progesterone, soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), mid-regional pro-atrial natriuretic peptide (MR-proANP), copeptin (CT-proAVP), and NfL were measured in 56 women with complete clinical data.

Results: Placental delivery significantly decreased all placental markers: progesterone 4.5-fold, PlGF 2.2-fold, and sFlt-1 1.7-fold. Copeptin and MR-proANP increased slightly (1.4- and 1.2-fold, respectively). Unexpectedly, NfL levels (median [interquartile range]) increased significantly post-partum: 49.4 (34.7–77.8) vs 27.7 (16.7–31.4) pg/ml ($p < 0.0001$). Antepartum NfL was the sole independent predictor of NfL peri-partum change; mode of delivery, duration of labor, clinical characteristics and other biomarkers were all unrelated. Antepartum NfL levels were themselves independently predicted only by maternal age.

Conclusions: Parturition per se increases maternal serum NfL levels, suggesting a possible impact of parturition on maternal neuronal integrity.

BMC Pregnancy and Childbirth

RESEARCH ARTICLE Open Access

Impact of parturition on maternal cardiovascular and neuronal integrity in a high risk cohort – a prospective cohort study

Katrina Suzanne Evers[1*†], Evelyn Annegret Huhn[2†], Sotirios Fouzas[3], Christian Barro[4], Jens Kuhle[4], Urs Fisch[4], Luca Bernasconi[5], Olav Lapaire[2†] and Sven Wellmann[1,6†]

Abstract

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Conclusions: Parturition per se increases maternal serum NfL levels, suggesting a possible impact of parturition on maternal neuronal integrity.

Keywords: Parturition, Brain, Delivery, Pregnancy, Birth, Surrogate marker

Background

Parturition triggers major multisystem changes, most notably hormonal and cardiovascular, which are as precipitate as those in pregnancy are progressive. However, we know little about the impact of parturition and pregnancy on maternal neuronal integrity. Despite some studies of neural change, including effects on brain size [1], neuronal morphology [2] and neuroplasticity [3], postpartum changes in the levels of specific biomarkers for maternal neuronal injury, stress and hemodynamics have not been systematically explored to date.

The scaffold of neurons is composed of certain proteins, including neurofilaments (Nf), which are highly specific major neuronal scaffolding proteins and which are composed by 4 subunits: the triplet of Nf light chain (NfL), Nf medium chain, and Nf heavy chain (NfH), and α-internexin in the central nervous system (CNS), or peripherin in the

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peripheral nervous system [4]. Neuronal damage, acute or chronic, leads to a release of Nf fragments into the extracellular fluid, cerebrospinal fluid (CSF) and peripheral blood [4–6]. Highly sensitive single molecule array (Simoa) immunoassay has improved NfL detection, particularly in peripheral blood, making NfL a promising and readily accessible biomarker for neuroaxonal injury even in very slowly progressing diseases such as Alzheimer's disease and before the onset of clinical symptoms [7].

Copeptin, a peptide derived from the same precursor as arginine vasopressin, is a robust and equally accessible biomarker for fluid equilibrium, vascular tone, and individual stress [8–10]. Mid-regional pro-atrial natriuretic peptide (MR-proANP), a stable by-product of atrial natriuretic peptide, is an established biomarker for hemodynamic stress and hypertension [11]. Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1), which both derive from the placenta, are biomarkers for preeclampsia with PlGF as one of the most highly modulated maternal blood proteins during the gestational age span [12, 13]. Furthermore the ratio of PlGF/sFlt-1 is a marker for the burden of placental lesions consistent with uteroplacental underperfusion [14].

Our aim was to use these biomarkers to advance understanding of the physiological changes that occur in the maternal system after delivery.

Methods

The study was nested within a prospective cohort study conducted at the University Hospital of Basel between 2012 and 2015 [15–17]. Following approval by the Northwest Switzerland Ethics Committee (PB_2016–02490), written informed consent was obtained from all participants. The above mentioned prospective cohort study focused on the diagnostic accuracy of biomarker cut-off values in the assessment of preeclampsia. Throughout the study a subgroup study was performed which focused on the postpartal course that resulted in the indicated study number of 56 patients with paired ante- and postpartum blood samples. Women were included when they were aged > 18 years with a singleton pregnancy and presented at least one risk factor for preeclampsia such as obesity with a body mass index (BMI) > 26.1 kg/m^2, age > 40 years, preexisting or gestational diabetes mellitus, essential hypertension or renal disease, pregnancy-induced hypertension, uteroplacental dysfunction, previous preeclampsia, eclampsia or HELLP Exclusion criteria were chromosomal aberrations and fetal malformations, abortion, or stillbirth < 22 weeks of gestation. Demographic characteristics and medical history were recorded prospectively, and serum samples were obtained one day before and one day after parturition.

Antecubital blood samples were processed using a standardized procedure, consisting in transfer to a central laboratory, centrifugation, preparation of serum aliquots, and storage at − 80 °C until analysis. No sample had previously been thawed. Assay staff were blinded to patients' clinical information and pregnancy outcome.

Serum sFlt-1 (pg/ml) and PlGF (pg/ml) were measured by Roche Elecsys assay on two electrochemiluminescence immunoassay platforms: Modular E170 (Roche Diagnostics, Rotkreuz, Switzerland) to October 2014 and Cobas 6000 (Roche Diagnostics) from November 2014 to study end [18]. For quality control samples the within-run coefficient of variation was below 1.5% for the sFlt-1 and below 0.9% for the PlGF assay on Modular E170. Between-run coefficients of variation were 2.5 to 3.9% for the sFlt-1 and 2.7 to 3.7% for the PlGF assay on Modular E170 and 1.2 to 2.3% for the sFlt-1 and 1.7 to 2.0% for the PlGF assay on the Cobas 6000 platform.

NfL (pg/ml) was determined by Simoa assay as previously described [19, 20].

MR-proANP (pmol/l) and copeptin (pmol/l) were measured in a single batch using fully automated BRAHMS KRYPTOR assays (B·R·A·H·M·S GmbH, part of Thermo Fisher Scientific, Hennigsdorf, Germany) [16].

Progesterone (pg/ml) was measured by ELISA kit (Enzo Life Sciences, Inc., Farmingdale, New York) according to the manufacturer's protocol.

Statistical analysis

Continuous variables are presented as median with interquartile range, and categorical variables as number of cases and percentages. Ante- vs postpartum biomarker changes were assessed using the non-parametric Wilcoxon matched-pairs signed rank test. Ante- and postpartum relationships between biomarkers were assessed by Spearman's correlation and displayed in a heat map: individual coefficients (Spearman's rho) were presented in a matrix as different color gradients, from blue (absolute positive correlation: coefficient 1) through red (absolute negative correlation: coefficient − 1). Linear regression analyses (univariable and multivariable models) explored the determinants of each biomarker change after delivery (calculated as the postpartum value/antepartum value ratio and log transformed) and was performed in two steps: a) single (exploratory) regression, in which the effect of each parameter was assessed separately, and b) multivariable modeling, in which only parameters with statistical significance $P < 0.100$ in the exploratory analysis were included in the multivariable model. Statistical analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, New York; RRID:SCR_002865).

Results

The study enrolled 56 women with paired ante- and postpartum serum samples (Fig. 1) and complete clinical

and biomarker data (Table 1). Comparison between ante- and postpartum values (Table 2) showed that placental markers (progesterone, sFlt-1, and PlGF) decreased postpartum as expected, whereas cardiovascular (stress-related) biomarkers MR-proANP and copeptin, and neuronal injury marker NfL, increased significantly (Fig. 2). The relative change (prepartum to postpartum ratio) of each parameter is presented in Fig. 3 and the exact values in the supplement (Additional file 1: Table S1). The specific correlations between these biomarkers before and after delivery are presented in Fig. 4.

As anticipated our experiments demonstrated that the angiogenesis-related biomarkers sFlt-1 and PlGF showed an inverse relationship and that sFlt-1 was higher and PlGF was lower in PE, both antepartum and postpartum (sFlt-1 antepartum: 8.999 (7433–13, 082) pg/ml vs. 4254 (3045–6671) pg/ml; $P < 0.001$ and sFlt-1 postpartum: 5341 (2644–7225) pg/ml vs. 3017 (1647–3834) pg/ml; $P = 0.002$; PIGF antepartum: 83 (65–142) pg/ml vs. 164 (86.5–158.5) pg/ml; $P = 0.014$; PIGF postpartum: 40 (28–60) pg/ml vs. 64 (38–107) pg/ml; $P = 0.033$). Upon closer examination of the biomarkers' change we evaluated a sFlt-1 ratio of 0.52 (0.42–0.68) with PE vs. 0.60 (0.47–0.75) without PE ($p = 0.268$) and a PIGF ratio of 0.45 (0.29–0.97) with PE vs. 0.43 (0.29–0.68) without PE ($p = 0.559$).

Linear regression analyses exploring the determinants of postpartum biomarker change are presented as supplements (Additional file 1: Tables S2-S7). In brief, except for sFlt-1, antepartum levels were the strongest predictor of individual biomarker change. In particular, the higher the levels of progesterone and PlGF antepartum, the greater the decrease postpartum. In contrast, the lower the antepartum levels of copeptin, MR-proANP, and NfL, the greater their postpartum increase. Change in MR-proANP was also independently determined by maternal age, while that in sFlt-1 was influenced by the change in hemoglobin. In addition, the younger the mother, the lower the antepartum NfL. Although NfL increased after delivery in 49 women and decreased in the remainder, the two groups did not differ significantly in clinical characteristics (Additional file 1: Table S8). Moreover, vaginal delivery subgroup analysis showed no correlation between ante- or postpartum biomarker levels and the duration of either the first or second stage of labor (data not shown).

Discussion

In this prospective study we showed that the progesterone and angiogenic biomarkers PlGF and sFlt-1 decrease after delivery, whereas stress marker copeptin and heart failure marker MR-proANP increase. However, the key finding was that neuronal injury marker NfL increases postpartum, independently of clinical variables or other biomarkers.

Maternal serum progesterone plunges after placental delivery [21], initiating profound endocrine adaptations, including onset of lactation [22] and reversal of the pregnancy-induced changes in the angiogenic system, with a return to non pregnant PlGF and sFlt-1 levels [23]. Negative correlation between PlGF and sFlt-1 is well documented: as term approaches the relationship becomes progressively reciprocal,

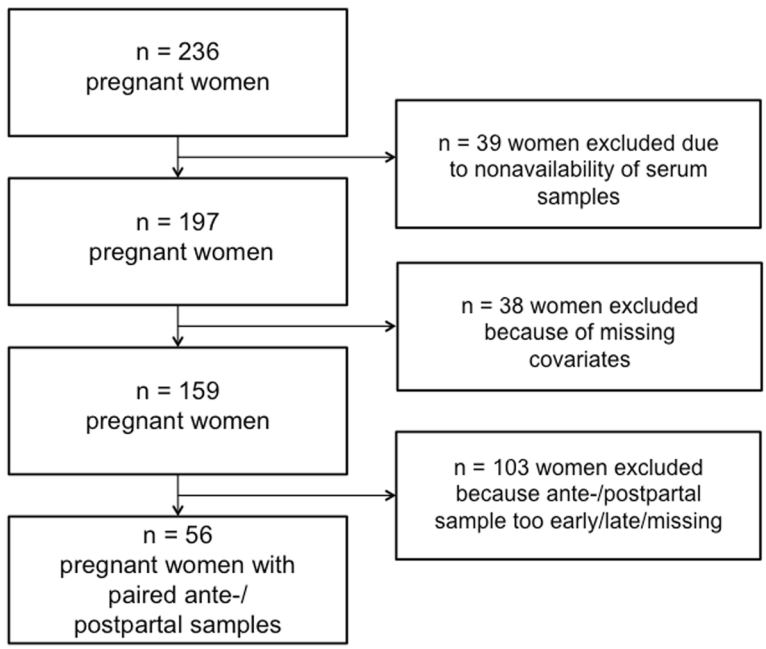

Fig. 1 Study flow chart

Table 1 Baseline characteristics of the study cohort

Maternal characteristics	
Age, years	32.6 ± 4.8 (23–45)
BMI, kg/m^2	31.9 ± 6.4 (23.0–49.5)
Parity	
1	37 (66.1)
2+	19 (33.9)
Gestation and delivery	
Gestational age, weeks	38.1 ± 2.3 (32.3–41.3)
Prematurity	15 (26.8)
Gestational diabetes	17 (30.4)
- Insulin-dependent	9 (52.9)
- Lifestyle management	8 (47.1)
Preeclampsia	21 (37.5)
- Early-onset	4 (19)
- Late-onset	17 (81)
- Medical treatment	16 (76.2)
- Labetalol	16 (100)
- Methyldopa	1 (6.2)
- Nifedipine	1 (6.2)
- Magnesium	16 (100)
Delivery mode	
Vaginal	31 (55.4)
Cesarean section	25 (44.6)
- Primary	16 (28.6)
- Secondary	9 (16.1)
Epidural anesthesia	51 (91.1)
Narcotics for pain during labor	0 (0)
Neonatal characteristics	
Male sex	29 (51.8)
Birth weight, g	2893 ± 702 (1150–4095)
Birth length, cm	47.3 ± 3.8 (35–54)
Apgar score (5 min)	9.1 ± 1.1 (5–10)
pH (umbilical blood)	7.27 ± 0.5 (7.14–7.39)
Blood sampling	
Sampling during active labor	18 (32.1)
Before birth, hours	79.3 ± 21.6 (0.5–797)
After birth, hours	12.1 ± 1.9 (2.1–97)

Data are mean ± SD (range) or number of cases (%)

Table 2 Key ante- and postpartum parameters

	Antepartum	Postpartum	*p*-value
SBP, mmHg	145 (127–158)	142 (131–153)	0.9777
DBP, mmHg	80 (74–89)	80 (69–85)	0.4839
Hemoglobin, g/l	128 (122–132)	115 (106–125)	< 0.0001
Progesterone, pg/ml	254 (207.9–318)	59.6 (35.5–83.1)	< 0.0001
Copeptin, pmol/L	6.2 (3.4–8.3)	7.2 (5.3–15.6)	0.0003
MR-proANP, pmol/L	85.5 (58.4–141)	101.5 (65.6–154)	0.0059
NfL, pg/ml	27.7 (16.7–31.4)	49.4 (34.7–77.8)	< 0.0001
PlGF, pg/ml	113 (68.5–184)	53 (35.5–102.5)	< 0.0001
sFlt-1, pg/ml	7803 (4817–11,985)	4083 (2433–5822)	< 0.0001

Data presented as median (interquartile range). Statistical significance was assessed by Wilcoxon's matched-pairs signed rank test
SBP systolic blood pressure, *DBP* diastolic blood pressure, *MR-proANP* by-product of atrial natriuretic peptide, *NfL* neurofilament light chain, *PlGF* placental growth factor, *sFlt-1* soluble fms-like tyrosine kinase-1

with lower free PlGF levels and rising levels of total sFlt-1, especially in preeclampsia [12]. This contrasts with the positive correlation between the rising levels of progesterone and MR-proANP as gestation advances [24]. Nevertheless it is crucial to note that PE did not affect the direction of change of both sFlt-1 and PlGF – both biomarkers decreased after parturition, in a way similar to that of normotensive women.

Vaginal delivery in particular is stressful, as reflected in the postpartum increases in the stress marker copeptin and cardiac marker MR-proANP [25, 26]. However, mode of delivery was not a significant determinant of either biomarker in our cohort, perhaps due to the blood sampling time points and short biomarker half-lives (≈60 min each, measured in non-pregnant individuals) [8, 27]. In a previous study blood samples were collected around 30 min after birth in contrast to the average of 13 h after birth in our study [26]. Our group along with others previously stated, that MR-proANP can represent a supplement to the well-established biomarkers and can support diagnosis of PE at triage [16, 28]. The biomarker reflects cardiovascular hemodynamic stress, arterial stiffness and may display the severity of hypertension [11]. Despite the fact that the N-terminal pro B-type natriuretic peptide (NT-proBNP) is considered as the Gold Standard biomarker in heart failure MR-proANP emerges as a valuable biomarker for the prediction of death and heart failure related events in patients with hypertrophic cardiomyopathy and has shown similar diagnostic performance when compared with NT-proBNP [29–31]. Although MR-proANP shows significant associations with indexes of target organ damage, its ability to discriminate between normal and "abnormal" indexes of heart failure or peripheral arterial disease such as ankle-brachial index, urinary albumin creatinine ratio or left ventricular mass index is relatively modest [11]. Published data on peripartal measurement of hemodynamics are scarce due to the limited evaluation methods with rarely performed invasive methods and the difficulty of continuous monitoring with noninvasive methods [32, 33]. Nevertheless one study focused on hemodynamics immediately after vaginal delivery in healthy pregnant women and noted a significant increase in heart rate, stroke volume and cardiac output at the time of newborn delivery compared with the baseline value measured at the onset of labor with the heart rate decreasing to baseline ten minutes after birth whereas stroke volume and

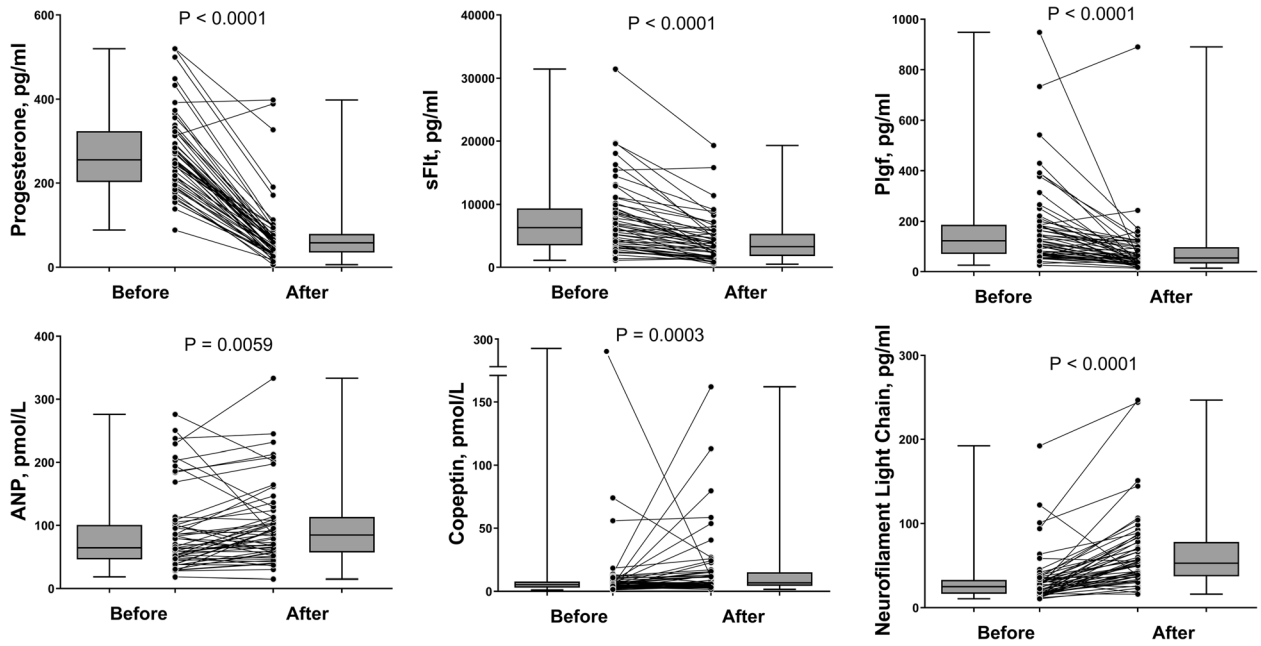

Fig. 2 *Biomarker changes before and after delivery.* Statistical significance was assessed by the Wilcoxon matched-pairs signed rank test. sFlt-1: soluble fms-like tyrosine kinase-1, PlGF: placental growth factor, MR-proANP: by-product of atrial natriuretic peptide

cardiac output decreasing but remaining higher that at labor onset until 120 min after delivery. The authors interpret this as a temporary increase in the circulating blood volume by transfusion from the uterus and/or release of inferior vena cava compression associated with uterine contractions [34]. To our knowledge the association of cardiac biomarkers and ventricular function has so far not been investigated around delivery but in pregnancies complicated by pregnancy-induced hypertension, where an impaired systolic function accompanied by an elevation of NT-proBNP levels were detected [35]. We therefore suggest that the relative change of MR-proANP of 1.28 in our study might also be due to the strain on the heart by the abovementioned increase in cardiac output occurring right after delivery.

The only identifiable factor affecting postpartum NfL was antepartum NfL, for which the sole identifiable determinant was maternal age: levels were lower in younger women. Increased NfL showed no association with either clinical characteristics or other biomarkers. We therefore speculate that the increase is triggered by parturition per se. This could be consistent with the incrimination of the long-lasting oxidative and/or psychogenic stress associated with vaginal delivery in the 2-fold increase in postpartum serum levels of glial-specific S100

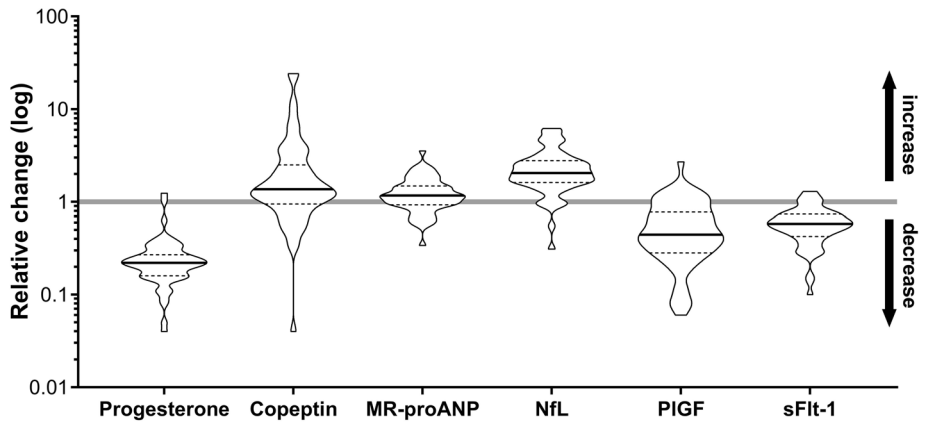

Fig. 3 *Relative change (antepartum to postpartum ratio) of key biomarkers.* Values are presented as "violin" plots showing both the range and density of the distribution. The line of equality (no change) is also depicted

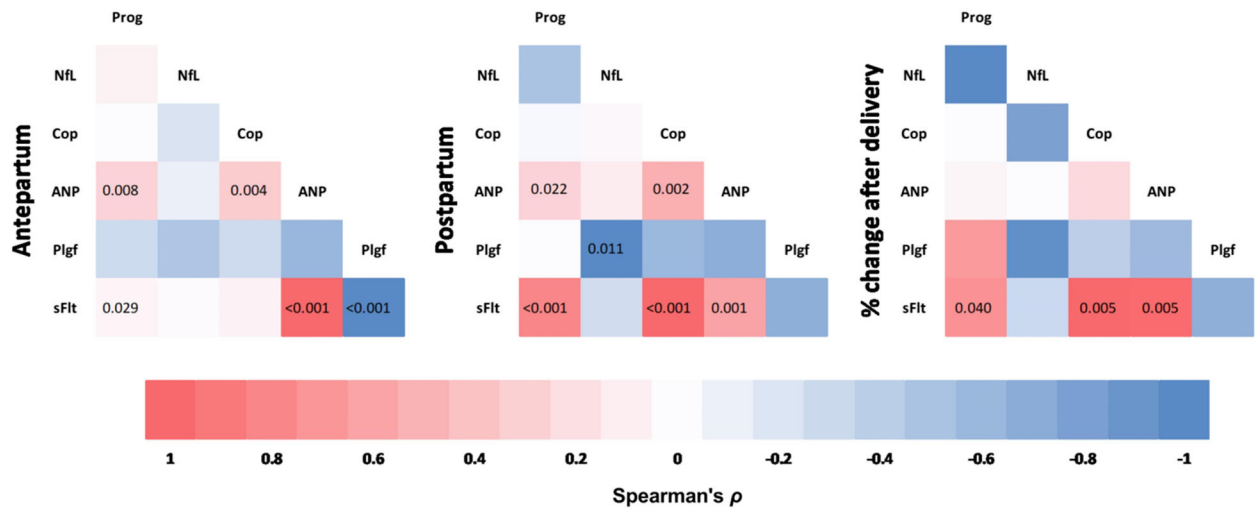

Fig. 4 *Heat map showing the level of correlation between biomarkers before and after delivery.* Correlation coefficients (Spearman's rho) are presented as different color gradients, from red (absolute positive correlation: correlation coefficient 1) to blue (absolute negative correlation: correlation coefficient – 1). The numbers in the cells represent the correlation *p* value (only significant *p* values are shown)

calcium-binding protein B seen in women giving birth spontaneously vs those undergoing elective cesarean section [36]. Although no difference has been reported in nerve growth factor levels between pregnancy and one week postpartum [37], it should be borne in mind that nerve growth factor is a neurotrophic marker rather than a marker of neuronal damage.

Generally, acute or chronic neuroaxonal damage elevates serum NfL levels via potentially three different mechanisms: (i) neuronal destruction in the central or peripheral nervous system, (ii) increased blood-brain barrier (BBB) permeability, and (iii) increased neuronal turnover [38]. The latter is a rather unlikely explanation for the peripartum increase in NfL, but parturition may affect neuronal integrity by impairing BBB permeability. In animal studies elevated levels of vascular endothelial growth factor (VEGF) increase BBB permeability during pregnancy via complex interaction between VEGF and its two receptors but so far the role of VEGF on the BBB is not known in human pregnancy and preeclampsia [39].

In addition, we and others recently showed that NfL levels increase during pregnancy, above all in women at risk of, or with early signs of, preeclampsia [18, 40]. Recent data show focal volume reduction in gray matter in first-time mothers persisting for at least two years postpartum [41]. Whether increased postpartum NfL is associated with these structural brain changes remains to be determined.

Interestingly, postoperative NfL levels in non-pregnant surgical patients increase significantly over preoperative values, in a range very similar to the almost 2-fold increase we identified, suggesting that general anesthesia and surgery are associated with at least short-term neuronal damage [42, 43]. Given that none of our women underwent general anesthesia but 91% had epidural anesthesia and that NfL levels did not differ between women delivering spontaneously compared to those delivering by caesarean we conclude that giving birth per se has a negative impact on neuronal integrity.

We cannot exclude the possibility that in addition to the central nervous system the peripheral nervous system or other tissues also contributes to the increase in NfL. For example, the human uterus is a highly innervated muscle with abundant adrenergic and cholinergic fibers [44] and its involution after delivery may be accompanied by axon destruction. However, to the best of our knowledge, neuroaxonal injury in the postpartum uterus has not yet been reported and therefore remains speculative. Furthermore we cannot rule out that alternative sources such as the thyroid, adrenal or parathyroid gland which all feature ribonucleic acid (RNA) expression or adipose and soft tissue where NfL protein expression can be detected (data available from v19.proteinatlas.org) and which are much affected tissues during labor play a role in the increased NfL concentrations [45]. Another potential source is the fetal or placental compartment. As far as we currently know the placenta has not yet been investigated in relation to NfL. Since the molecular size of NfL has a size of around 60–70 kDa and the human placenta is freely permeable to solutes of 1350–5200 Da we do not believe that the fetus is a probable source for NfL [46–48]. Once again we also want to point out, that our cohort is a group of pregnant women at high risk to develop preeclampsia of which

some also show conditions such as diabetes. To what extent these co-morbidities play a role in comparison has to be the subject of further studies.

A major limitation of our study is on the one hand the lack of blood samples from additional time points to explore the dynamics of NfL and other biomarkers over a longer period before and after delivery and on the other hand the absence of CSF samples to be able to validate the intracerebral origin of NfL.

Conclusions

In summary, our study characterizes the evolution of various cardiovascular and neuronal serum biomarkers from before to after parturition in a cohort at high risk of developing preeclampsia and shows for the first time that maternal serum NfL levels increase postpartum independently of delivery mode, gestational age and other clinical parameters. Additional studies are needed to verify the hypothesis that parturition per se has an impact on maternal neuronal integrity.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12884-019-2570-6.

Additional file 1: Table S1. Relative antenatal - postnatal change in key parameters**. Table S2.** Relationships of Copeptin change (log) after delivery. **Table S3.** Relationships of MR-proANP change (log) after delivery. **Table S4.** Relationships of NfL change (log) after delivery. **Table S5.** Relationships of PlGF change (log) after delivery **Table S6.** Relationships of sFlt-1 change (log) after delivery. **Table S7.** Determinants of NfL levels (log) before delivery. **Table S8.** Comparisons between cases in which NfL increased and those in which NfL decreased after delivery.

Abbreviations

BBB: Blood-brain barrier; BMI: Body mass index; CNS: Central nervous system; CSF: Cerebrospinal fluid; CT-proAVP: C-terminal pro-arginine vasopressin; ELISA: Enzyme-linked immunosorbent assay; HELLP: Acronym for hemolysis-elevated liver enzymes low platelet count; kDa: Kilodalton; MR-proANP: Mid-regional pro-atrial natriuretic peptide; Nf: Neurofilaments; NfH: Neurofilament heavy chain; NfL: Neurofilament light chain; NT-proBNP: N-terminal pro B-type natriuretic peptide; pg/ml: Picograms per millilitre; PlGF: Placental growth factor; pmol/l: Picomoles per litre; RNA: Ribonucleic acid; RRID: Research Resource Identifiers; SD: Standard deviation; sFlt-1: Soluble fms-like tyrosine kinase-1; Simoa: Single molecule array; VEGF: Vascular endothelial growth factor

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Authors' contributions

EH protocol/project development, data collection/management; KE protocol/project development, manuscript writing/editing, data interpretation; OL data collection/management, protocol/project development, data interpretation; LB data analysis; CB data analysis; UF data analysis; SF data analysis, manuscript writing; JK data analysis, data interpretation; SW protocol/project development, data interpretation, manuscript writing/editing. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was nested within a prospective cohort study conducted at the University Hospital of Basel between 2012 and 2015. Following approval by the Northwest Switzerland Ethics Committee (PB_2016–02490), written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Manuscript 3: Serum Neurofilament Levels in Children With Febrile Seizures and in Controls

Journal: Frontiers in Neuroscience – published

Authors: Katrina S. Evers, Melanie Hügli, Sotirios Fouzas, Severin Kasser, Christian Pohl, Benjamin Stoecklin, Luca Bernasconi, Jens Kuhle and Sven Wellmann

Abstract

Objective: Neuroaxonal damage is reflected by serum neurofilament light chain (sNfL) values in a variety of acute and degenerative diseases of the brain. The aim of this study was to investigate the impact of febrile and epileptic seizures on sNfL, serum copeptin, and prolactin levels in children compared with children with febrile infections without convulsions.

Methods**:** A prospective cross-sectional study was performed in children aging 6 months to 5 years presenting with fever (controls, *n* = 61), febrile seizures (FS, *n* = 78), or epileptic seizures (ES, *n* = 16) at our emergency department. sNfL, copeptin, and prolactin were measured within a few hours after the event in addition to standard clinical, neurophysiological, and laboratory assessment. All children were followed up for at least 1 year after presentation concerning recurrent seizures.

Results**:** Serum copeptin values were on average 4.1-fold higher in FS and 3.2-fold higher in ES compared with controls (both $p < 0.01$). Serum prolactin values were on average 1.3-fold higher in FS compared with controls ($p < 0.01$) and without difference between ES and controls. There was no significant difference of mean sNfL values (95% CI) between all three groups, FS 21.7 pg/ml (19.6–23.9), ES 17.7 pg/ml (13.8–21.6), and controls 23.4 pg/ml (19.2–27.4). In multivariable analysis, age was the most important predictor of sNfL, followed by sex and C reactive protein. Neither the duration of seizures nor the time elapsed from seizure onset to blood sampling had an impact on sNfL. None of the three biomarkers were related to recurrent seizures.

Significance**:** Serum neurofilament light is not elevated during short recovery time after FS when compared with children presenting febrile infections without seizures. We demonstrate an age-dependent decrease of sNfL from early childhood until school age. In contrast to sNfL levels, copeptin and prolactin serum levels are elevated after FS.

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Serum Neurofilament Levels in Children With Febrile Seizures and in Controls

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Keywords: neuronal biomarker, convulsion, epilepsy, neurofilament, paroxysmal

INTRODUCTION

Febrile seizures (FS) are the most common convulsive events in children aged between 6 months and 5 years and arise in 2 to 5% of all children. FS are defined as seizures occurring during childhood associated with fever that is not caused by an infection of the central nervous system (Subcommittee on Febrile Seizures American Academy of Pediatrics, 2011). FS are classified as simple or complex seizures depending on age at onset, duration, short-term recurrence, and type of seizure (Livingston et al., 1979). In approximately one third of children with a first febrile seizure, a second episode, and in around 10%, three or more FS will occur (Berg et al., 1997). Especially prolonged FS may be associated with substantial long-term neurological morbidities such as temporal lobe epilepsy or mesial temporal sclerosis with possible subsequent intellectual disability (Pujar et al., 2018).

Prolactin is a polypeptide hormone secreted by the anterior pituitary gland but also in other tissues and organs such as adipose tissue, uterus, and immune cells. Apart from the production of milk, prolactin is also known to play a role in the regulation of the immune system, behavior, and metabolism. Initially, Trimble et al. debated that seizures could raise prolactin levels (Trimble, 1978). In the past decades, it has gained recognition in the support of the diagnosis of epileptic seizures in particular for the differentiation of generalized tonic–clonic or complex partial seizures from psychogenic non-epileptic seizures among adults and older children especially when the clinical setting does not provide video-EEG recording (Chen et al., 2005; Abubakr and Wambacq, 2016; Fisher, 2016).

Another hormone released by the pituitary gland is arginine vasopressin (AVP) which plays a major role not only in maintaining the fluid balance and vascular tonus but also in the regulation of the endocrine stress response. Copeptin derives from the same precursor molecule, is more stable, and is released into the periphery in the same ratio as AVP (Evers and Wellmann, 2016). Published data suggest that copeptin is involved in the thermoregulatory response to fever and convulsions and copeptin has lately been shown to have high diagnostic accuracy in FS (Kasting et al., 1980, 1981; Landgraf et al., 1990; Stocklin et al., 2015).

Neurofilaments (Nf) are highly specific major scaffolding proteins of neurons consisting of four subunits: the triplet of NfL (Nf light), Nf medium, and NfH (Nf heavy) chains and alpha-internexin in the CNS, or peripherin in the peripheral nervous system (Teunissen and Khalil, 2012). Disruption of the axonal cell membrane due to acute or chronic neuronal damage releases Nf into the interstitial fluid and eventually to the cerebrospinal fluid (CSF) and the blood compartment (Khalil et al., 2018).

Matsushige and colleagues recently determined serum pNF-H (phosphorylated form of neurofilament-heavy chain) levels in patients with prolonged and simple FS to evaluate neuronal damage and were able to show that serum pNF-H levels in children with prolonged FS were significantly higher than in children without FS (Matsushige et al., 2012). Shahim et al. (2013) demonstrated that CSF NfL levels in children were increased in status epilepticus compared with unspecified epilepsy and that NfL levels were significantly higher in lysosomal and mitochondrial disorders than in neurodegenerative disorders without known etiology. Higher NfL levels in children with suspected multiple sclerosis are predictive for clinically definite multiple sclerosis diagnosis (van der Vuurst de Vries et al., 2018; Wong et al., 2019). Furthermore, CSF NfL levels had the highest capability to distinguish opsoclonus–myoclonus syndrome from controls compared with other brain cell–specific biomarkers in a pediatric cohort (Pranzatelli et al., 2014).

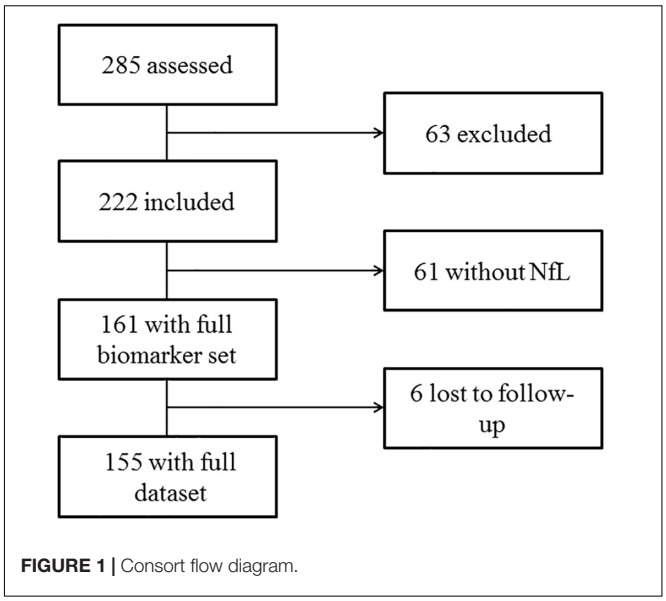

FIGURE 1 | Consort flow diagram.

The aims of this study were (1) to evaluate the short-term impact of convulsions on serum NfL (sNfL) levels in a cohort of children presenting with FS in comparison with children with febrile infections and epileptic seizures at an emergency department (ED); (2) to compare sNfL levels with other postictal serum biomarkers, namely, copeptin and prolactin; and (3) to characterize sNfL levels in the population of young children in general.

MATERIALS AND METHODS

The study was based on data and blood samples prospectively collected from a child cohort established at the University Children's Hospital of Basel (UKBB), Switzerland, between May 2013 and November 2015. The Cantonal Ethics Committee of Basel approved the study protocol (EK352/12), and written informed consent was obtained from the parents. The study was registered in the clinical trial registry ClinicalTrials.gov (No. NCT01884766). Information concerning eligibility criteria and the inclusion procedure can be obtained elsewhere (Stocklin et al., 2015). Serum concentrations of NfL were determined with a Simoa assay, which was established using the NF-light assay ELISA kit from UmanDiagnostics (Umeå, Sweden), transferred onto the Simoa platform with a homebrew kit (Quanterix, Boston, MA, United States), and has been described in detail by our group previously (Disanto et al., 2017). Calibrators (neat) and serum samples (1:4 dilution) were

TABLE 1 | Characteristics of the study groups.

	Controls (n = 61)	Febrile seizures (n = 78)	Epileptic seizures (n = 16)
Males/females	33/28	43/35	10/6
Age, months	29.4 ± 17.8 (6–72)	24.8 ± 14.5 (6–63)	53.9 ± 45.8 (9–163)*
Body weight, kg	12.9 ± 4.2 (6.8–27)	12.1 ± 3.5 (6.0–23.0)	18.4 ± 13.0 (4.4–56)†
History of seizures	NA	16 (20.5)	9 (56.3)‡
Temperature at home, °C	39.6 ± 0.7 (37.7–41.3)	39.3 ± 0.6 (38.0–41.0)	NA
Temperature at ED, °C	38.3 ± 1.0 (36.0–40.5)	38.6 ± 0.8 (36.5–40.1)	NA
Duration of event, min	NA	6.5 ± 8.1 (1–40)	5.1 ± 5.2 (1–20)
Time to presentation, min	NA	107 ± 70.7 (1–330)	96.2 ± 60.9 (7–240)§
Laboratory data at ED			
Hct, %	35.6 ± 3.7 (27.3–43.3)	37.2 ± 4.2 (28.9–56.0)	38.2 ± 3.3§§(31.8–42.3)§
WBC × 1000/mm^3	12.4 ± 7.2 (1.9–40.8)	12.9 ± 7.1 (3.4–34.2)	8.6 ± 2.7 (5.2–14.7)
Na, mmol/L	136.1 ± 3.2 (129–142)	135 ± 2.9 (118–141)	138 ± 2.1 (135–143)
Cl, mmol/L	105 ± 3.2 (98–112)	105 ± 2.6 (98–112)	106 ± 2.2 (101–110)
pH	7.37 ± 0.05 (7.20–7.40)	7.36 ± 0.06 (7.20–7.50)	7.27 ± 0.07** (7.10–7.30)**
CO_2, mmHg	31.6 ± 4.4†† (21–41)††	33 ± 5.1 (24–54)	43.2 ± 10.7 (34–70)
Bicarbonate, mmol/L	21.7 ± 2.6 (13.9–26.1)	21.6 ± 1.6 (17.5–25.3)	21.5 ± 2.6 (14.9–25.2)
Lactate, mmol/L	1.5 ± 0.8 (0.9–4.5)	1.5 ± 0.7 (0.7–4.5)	1.2 ± 0.5 (0.6–2.2)
CRP, mg/dl	50.5 ± 50.2 (0.3–220)††	12.6 ± 18.6 (0.3–91)	1.3 ± 2.4 (0.3–8.0)

*Data are presented as mean ± SD (range) unless stated otherwise. *p = 0.042 vs controls and p = 0.002 vs febrile seizures. †p = 0.043 vs. febrile seizures. ‡p = 0.010 vs febrile seizures. §p = 0.755 vs febrile seizures. §§p = 0.039 vs controls. ¶p = 0.002 vs febrile seizures. **$p < 0.001$ vs febrile seizures and controls. ††$p < 0.001$ vs febrile and epileptic seizures. Between-group comparisons were performed with Mann–Whitney U-test, Kruskal–Wallis one-way ANOVA test (with Bonferroni correction for multiple comparisons), χ^2 test, or Fisher's exact test, as appropriate. ED, emergency department; NA, not available; WBC, white blood cell.*

TABLE 2 | Differences in biomarkers among study groups.

	Controls (n = 61)	Febrile seizures (n = 78)	Epileptic seizures (n = 16)
sNfL, pg/ml	23.4 (19.2–27.4)	21.7 (19.6–23.9)	17.7 (13.8–21.6)
Prolactin, mU/L	320 (277–362)*	411 (365–458)*	429 (266–592)
Copeptin, pmol/L	9.7 (6.4–12.9)†,‡	39.9 (26.1–53.8)†	30 (13.7–46.2)‡

*Data are presented as mean (CI). *p = 0.012 for febrile seizures vs controls. †$p < 0.001$ for febrile seizures vs controls. ‡p = 0.002 for epileptic seizures vs controls. Between-group comparisons were performed with Kruskal–Wallis one-way ANOVA test (with Bonferroni correction for multiple comparisons).*

measured in duplicates. Bovine lyophilized NfL was obtained from UmanDiagnostics. Calibrators ranged from 0 to 2000 pg/ml. Batch prepared calibrators were stored at −80°C. Intra- and interassay variabilities of the assay were <10%. Repeated measuring was performed for the few samples with intra-assay coefficients of variation >20%.

Measurement of copeptin levels was done in a batch analysis with a commercial sandwich immunofluorescence assay (B·R·A·H·M·S Copeptin proAVP; Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany) as described in detail elsewhere (Morgenthaler et al., 2006). The lower detection limit of the copeptin assay was 0.69 pmol/L, and the functional assay sensitivity was <1 pmol/L.

Prolactin quantification was performed using the Roche Modular E 170 (Roche Diagnostics AG, Rotkreuz, Switzerland). The lower detection limit was 1 mU/L, and the inter-assay precision <3% coefficient of variance at 102, 450, and 816 mU/L, respectively.

Statistics

Statistical analyses were performed using SPSS for Windows version 24 (IBM, United States) and included descriptive statistics, Spearman's rank-order correlation analyses, and multiple linear regressions (MLR) using sNfL as dependent variable. sNfL variables were log10 transformed for the correlations and MLR. The independent variables included for MLR were based on significant correlations and significant non-parametric univariate analyses such as the one-way ANOVA test (with Bonferroni correction for multiple comparisons), Mann–Whitney U–test (2 levels), Kruskal–Wallis test (>2 levels), χ^2 test, or Fisher's exact test. The discriminatory ability of both copeptin and prolactin was assessed by receiver operating characteristic (ROC) curve analysis and was compared by means

TABLE 3 | Ability of biomarkers to diagnose seizures.

	All seizures (FS + ES vs controls)	Febrile seizures (FS vs controls)
sNfL	0.462 (0.370–0.555)	0.494 (0.396–0.592)
Prolactin	0.620 (0.529–0.710)	0.648 (0.554–0.741)
Copeptin	0.804 (0.733–0.875)	0.807 (0.733–0.882)

Data are AUC (95% CI). ES, epileptic seizures; FS, febrile seizures; sNfL, serum neurofilament light chain.

TABLE 4 | sNfL dependencies.

	Unadjusted effect			Adjusted effect			
	R^2	Beta	*p*-value	Model 1 (R^2 0.201)		Model 2 (R^2 0.301)	
				Beta	*p*-value	Beta	*p*-value
Seizures	0.013	−0.114	0.159				
Male gender	0.001	−0.027	0.736	0.232	0.035	0.300	0.005
Age	**0.165**	**−0.406**	**<0.001**	−0.337	0.002	−0.375	0.001
Body weight	**0.139**	**−0.373**	**<0.001**				
Temperature at home	0.012	0.110	0.235				
Temperature at ED	0.007	0.086	0.288				
Hct	0.011	−0.107	0.195				
WBC	0.002	−0.050	0.566				
Na	0.005	−0.069	0.437				
Cl	0.001	0.002	0.979				
pH	**0.051**	**0.227**	**0.010**				
CO_2	0.019	−0.138	0.121				
Bicarbonate	0.013	0.115	0.199				
Lactate	0.008	−0.088	0.327				
CRP	0.008	0.089	0.310	0.278	0.012	0.241	0.023
Prolactin	0.001	0.016	0.843				
Copeptin	**0.056**	**−0.237**	**0.003**			−0.318	0.003

The unadjusted effect of each parameter was calculated by simple linear regression analysis using sNfL values (after logarithmic transformation) as the dependent variable. Significant (p-value < 0.05) parameters of the unadjusted effect are displayed in bold. The adjusted effect was calculated by stepwise linear regression analysis. CRP, C reactive protein; ED, emergency department; sNfL, serum neurofilament light chain; WBC, white blood cell.

of the area under the curve (AUC). A *p*-value of <0.05 was considered statistically significant.

RESULTS

We recruited a total of 285 children from May 2013 until November 2015. After exclusion of 63 infants, a total of 222 children were included in the final analysis. Of these, 61 did not have enough material for the analysis of sNfL, resulting in complete biomarker sets of 161 children. Six children were lost to follow-up (**Figure 1**). The children's age varied between 6 and 163 months; 44% were female. We allocated 78 children to the FS group, 16 to the ES group, and 61 febrile children without seizures were defined as controls. The characteristics of all groups are presented in **Table 1**.

There was no significant difference in age, body weight, and temperature at home or at ED when comparing the controls with FS group; the ES group had overall slightly but not significantly higher values in age and body weight than the other groups (**Table 1**). Regarding the laboratory data, pH in the ES group was significantly lower compared with the FS and control groups, whereas the controls exhibited significantly higher C reactive protein (CRP) levels than the FS and ES groups (**Table 1**). In total, 16 children (20.5%) in the FS and nine children (56.3%) in the ES group had a history of previous convulsive events. Serum values of NfL, copeptin, and prolactin in the different study groups are summarized in **Table 2**. When comparing the biomarkers in accordance to presence of fever, mean sNfL levels (95% CI) were only slightly higher in children with fever than in children without fever [fever: 22.1 pg/ml (20.1–24.1), no fever: 21.6 pg/ml (17.8–25.4), $p = 0.017$]. The evaluation of impact of seizures on biomarker levels revealed that seizures did not affect the levels of sNfL [20.8 pg/ml (18.9–22.7) vs 23.6 pg/ml (19.5–27.7)], whereas prolactin was slightly elevated in children presenting with convulsions compared with children without seizures [415 mU/L (366–464) vs 320 mU/L (277–362)] and copeptin was significantly higher in the group with seizures compared with no seizures [37.0 pmol/L (26.0–48.0) vs 9.6 pmol/L (6.4–12.8), $p < 0.001$]. Of note, no differences were found between time to presentation, which is the time elapsed from event onset to presentation at the emergency department (**Table 1**). Because blood sampling was done in all patients with FS or ES upon presentation, there was also no difference in the time to sampling.

Receiver operating characteristic curve analysis revealed that the ability to diagnose seizures differed clearly between the individual biomarkers (**Table 3**) with copeptin demonstrating the highest AUC levels compared with prolactin and sNfL [FS + ES vs controls: copeptin 0.804 (0.733–0.875) pmol/L; prolactin 0.620 (0.529–0.710) mU/L; sNfL 0.462 (0.370–0.555) pg/ml]. In consideration of the finding that sNfL levels were higher in the presence of fever, we had a closer look at the relationship between sNfL and fever and were not able to detect a correlation (**Figure 2**). With respect to the type of FS, we could not find any differences between simple and complex FS in biomarker levels [simple FS: sNfL: 20.9 (19.0–22.8) pg/ml; prolactin: 415 (366–464) mU/L, copeptin: 37.8 (26.5–49.1) pmol/L; complex FS: sNfL: 23.6 (18.9–28.4) pg/ml, prolactin: 425 (354–496) mU/L, copeptin: 38.6 (22.9–54.2) pmol/L]. When appointing sNfL as a dependent

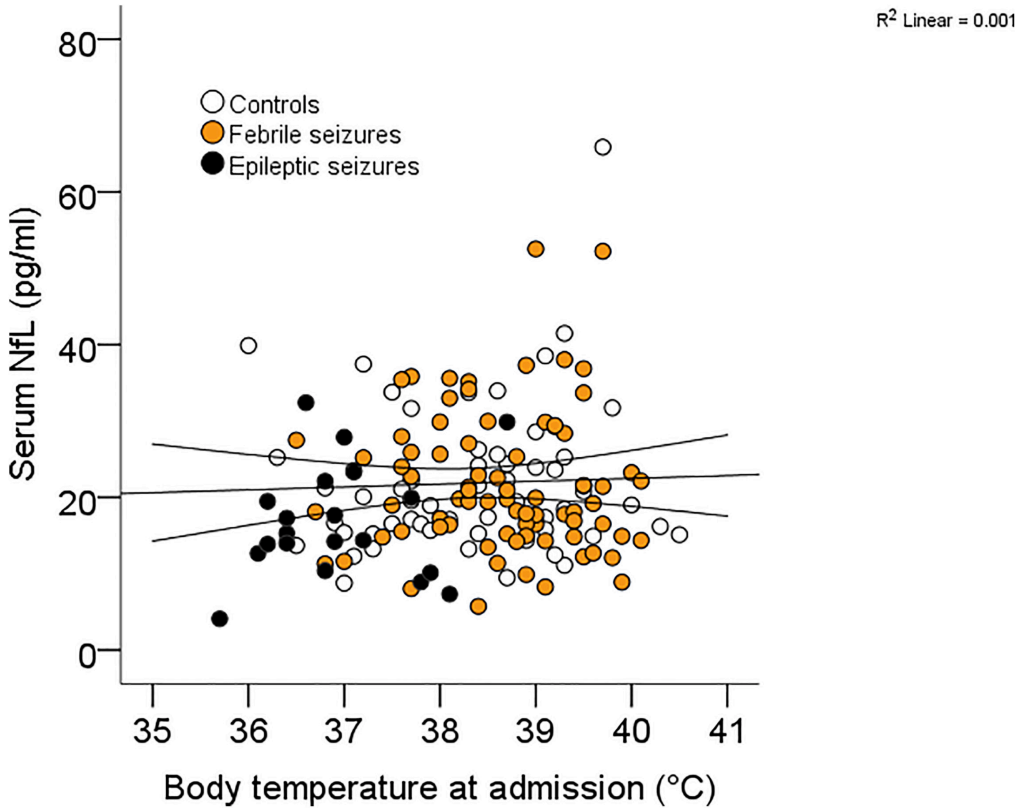

FIGURE 2 | Correlation between sNfL and body temperature.

variable in univariate models, sNfL had a significant inverse relationship with age and body weight (**Table 4**), indicating an age-dependent decrease of sNfL from early childhood until school age (**Figure 3**). MLR revealed age as the most important predictor of sNfL, followed by male sex and CRP. After including the two other biomarkers copeptin and prolactin into a model, also copeptin turned out to be a strong predictor for sNfL.

DISCUSSION

We prospectively investigated serum levels of NfL, copeptin, and prolactin in children presenting at an emergency department with FS, ES, or febrile infections without convulsions (controls). Our results provide evidence (1) that sNfL levels are not increased when measured within a few hours after convulsions in contrast to copeptin and prolactin levels; (2) that sNfL levels are higher in younger children, boys, and children with elevated CRP and elevated copeptin levels; and (3) that none of the three serum biomarkers are predictive for the recurrence of seizures.

The absent impact of convulsions on sNfL levels when measured a few hours after the events underlines the current state of evidence that simple FS are benign and do not increase the risk for the development of neurologic deficits (Steering Committee on Quality Improvement and Management, Subcommittee on Febrile Seizures American Academy of Pediatrics, 2008; Leaffer et al., 2013). Visser et al. (2012) also state that FS are not associated with problems in behavior or executive functioning in preschool children but did note an association of recurrent FS with an increased risk of expressive language delay at the age of 2.5 years which supports earlier findings about poorer language skills in school-aged children with a history of FS (Wallace, 1984). Matsushige et al. (2012) investigated the heavy chain of neurofilament (NfH) in serum of children suffering from febrile or epileptic seizures. The authors found a significant correlation between seizure duration and serum NfH levels during the first week in children with FS (Matsushige et al., 2012). Thus, whether sNfL levels may rise during recovery after febrile and epileptic convulsions warrants future studies.

Univariate analyses revealed a strong inverse relationship between sNfL and age and weight (**Table 4** and **Figure 3**). In multivariate analysis, for which weight was removed due to collinearity with age, age had the greatest impact on sNfL followed by male sex and CRP levels independently of seizures and fever. A very similar age dependency was described recently in a cohort of neurologically healthy children with decreasing sNfL in older children (Khalil et al., 2020; Reinert et al., 2020). In addition, between the age of 10 and 15 years, sNfL levels appear to mark a nadir, and beyond youth, sNfL levels increase in a linear fashion until the age of about 60 years. Afterward, sNfL levels were reported to rise much steeper

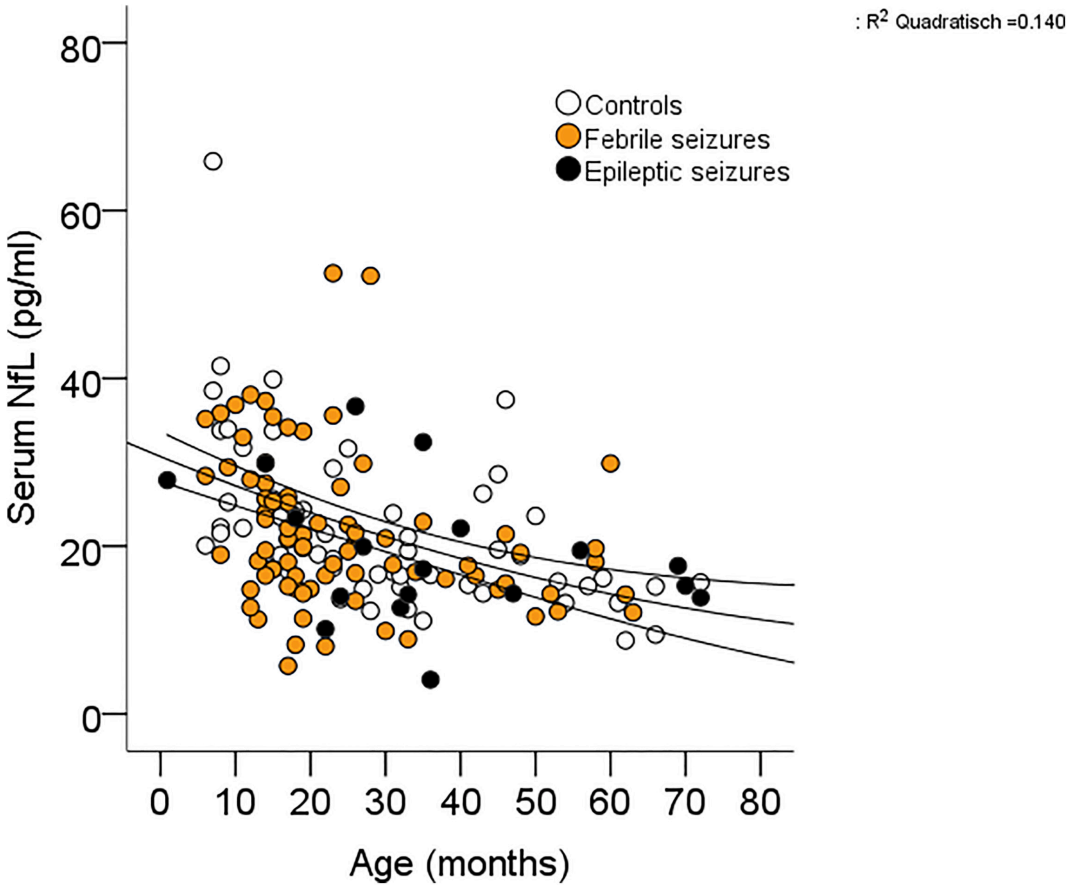

FIGURE 3 | Correlation between sNfL and age.

(Khalil et al., 2020; Reinert et al., 2020). Thus, considering sNfL level during the whole life cycle from high levels in newborn infants (Depoorter et al., 2018), decreasing until late childhood and then steadily increasing, sNfL levels represent a u-shaped curve. A possible explanation for high level in newborns is the developing brain with a high neuron turnover and a specialized system of tubulo-endoplasmic reticulum for protein transport. By the appearance of cerebral vessels being more fragile in infants than in adults, this might have the effect that the developing brain is more vulnerable (Saunders et al., 2012). In general, sNfL seems to reflect the substantial brain growth until adolescence followed by neuronal loss, which is associated with normal aging. Sexual disparity of biomarkers was described previously for copeptin in infants with higher levels in males. However, data on gender differences in sNfL are lacking (Burckhardt et al., 2014).

We observed that prolactin was elevated in the FS group when compared with the control group. The routine use of prolactin is not recommended due to limited accuracy. Moreover, copeptin levels were significantly higher in the FS group than in the control group and may be more useful for distinction of the underlying cause of the convulsive event (Stocklin et al., 2015; Pechmann et al., 2019). In contrast to these findings, our results could not provide additional support that copeptin and prolactin have the potential to predict upcoming convulsive events because none of the two biomarkers were related to recurrent seizures.

A few limitations need to be considered: the control group consisted of children presenting with febrile infections and our study revealed that sNfL levels are elevated in presence of fever alone and also correlate with CRP levels. This may lead to the suggestion that sNfL levels might be increased when compared with levels of healthy children without fever or contrariwise might only be elevated due to the rise in body temperature. We therefore propose to compare with a healthy afebrile cohort for verification of our hypothesis in potential upcoming studies. Furthermore, we must bear in mind that the diagnosis of a febrile seizure is solely based on the medical history and description of the caregivers; estimation by qualified personnel is therefore dependent on the statement of the accompanying parents. An overlap with simple shivering due to rise of temperature can therefore not be excluded. Besides, we merely analyzed blood samples at one timepoint; results of a further timepoint would give valuable information on the trend of sNfL levels and additionally might aid to assess the severity of suggested neuronal loss.

In conclusion, sNfL levels are not associated with febrile or epileptic seizures a few hours after the event, but significantly correlate with age, gender, and CRP. These findings are reassuring and indicate the benign nature of FS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Cantonal Ethics Committee of Basel. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SW, BS, KE, and JK conceived and designed the study. BS, SK, and CP were responsible for patient recruitment. JK and LB performed the biomarker measurements. SF performed the statistical analysis and prepared the tables and figures. SW, KE, MH, and JK interpreted the data. KE and SW drafted the initial manuscript. All authors critically revised the manuscript for important intellectual content, agreed on the final manuscript, and approved its submission for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Discussion

The aim of this PhD thesis was to evaluate promising novel biomarkers for their potential to support clinical assessment in perinatology and children presenting with febrile seizures. First we determined serum NfL concentrations in addition to the established preeclampsia biomarkers sFlt-1 and PlGF in pregnant women with high risk of preeclampsia, investigated clinical factors that influence serum NfL concentrations and examined the prognostic value of NfL compared with sFlt-1 and PlGF in predicting preeclampsia. Second we measured paired ante- and postpartum levels of cardiovascular, placental and neuronal injury markers in pregnant women at risk of developing preeclampsia to identify which impact birth *per se* has on several systems of the maternal body. Finally we explored the impact of febrile and epileptic seizures on NfL, copeptin and prolactin serum levels in children compared with with febrile infections without convulsions.

Current research

Our results show that NfL levels are increased in pregnant women at high risk of preeclampsia or with early signs of preeclampsia in comparison to those who did not and that NfL may have predictive value for preeclampsia particularly in older pregnant women. To our knowledge this present study was the first to measure the axonal injury serum marker in women at risk of developing preeclampsia. The findings show, that NfL may have predictive value for preeclampsia especially in older pregnant women. Since current risk assessment algorithms depend on clinical risk factors mostly unavailable for first-time pregnant women there is great need for novel, easily accessible biomarkers in order to deliver accurate preeclampsia risk assessment (Kenny et al., 2020). A Swedish group with a similar focus measured four different cerebral biomarkers in pregnant women and can support our results with showing an increase of NfL in the end of pregnancy in women developing preeclampsia in contrast to healthy pregnancies. Therefore the increase might reflect cerebral involvement before onset of disease (L. Bergman et al., 2018). Recently the same statement was made after increased NfL levels in CSF were determined in women with preeclampsia at delivery, with findings in MRI scans showing no difference in women with preeclampsia compared to women with normal pregnancies (Andersson et al., 2021). In contrast to this, the evaluation of the cerebral biomarker

neuron-specific enolase was not consistent in two studies and obtained contrary results with showing increased levels in the one group and decreased levels in the other group, both measured in women with preeclampsia (Andersson et al., 2021; Brzan Simenc, Ambrozic, Osredkar, Gersak, & Lucovnik, 2021). These findings strengthen our perspective that NfL may have the potential to serve as an early indicator of preeclampsia-induced neuroaxonal injury and might be able to enter clinical routine in future.

In regard to our findings of changes in maternal serum biomarkers after birth where we observed a decrease of angiogenic biomarkers in contrast to an increase of the stress marker copeptin and heart failure marker MR-proANP after delivery and notably determined that the neuronal injury marker NfL increased postpartum this may correspond to other previous published studies. Even though the number of publications dealing with the human brain in relation to pregnancy is limited, it is widely accepted that pregnancy and childbirth involves many maternal brain adaptations, which has mainly been evaluated by performing studies involving magnetic resonance imaging of maternal brains unlike our study focusing on serum NfL levels. Recently it was stated that first-time mothers show volume reductions of gray matter across pregnancy which last for at least 2 years postpartum (Hoekzema et al., 2017). Prior to these results a US-American group presented increased gray matter volumes comparing at 2-4 weeks postpartum with 3-4 months postpartum (P. Kim et al., 2010). These functional and structural cerebral changes are meant to be linked to the adaptation and preparation of women for emotional and cognitive demands needed for the care of a newborn child (Cárdenas, Kujawa, & Humphreys, 2019). The main driver of these adaptations seem to be pregnancy hormones in order for the woman to be ready for the organization of birth and the delivery of maternal care (Brunton & Russell, 2008). With the child's needs constantly changing with growing age, parents accordingly adapt to these needs, which results in a neural reorganization (Nithianantharajah & Hannan, 2006). Newest findings showed that the number of previous childbirths was negatively associated with white matter brain age, with the theory of parity having a protective effect on white matter later in life (Voldsbekk et al., 2021). So far biomarker studies comparing antepartum and postpartum levels have only been performed in women with preeclampsia showing persistent elevation of NSE and S100 calcium-binding protein B (S100B) levels 1 year after delivery concluding consistent cerebral involvement in preeclamptic women

(Lina Bergman et al., 2016). In our study mode of delivery, duration of labor, clinical characteristics and other biomarkers were all unrelated to the increase of NfL after birth with the only identifiable factor affecting postpartum NfL being antepartum NfL. Since increased NfL did not seem to be associated with clinical characteristics or other biomarkers we hypothesize that the increase is triggered by parturition itself.

Relating to the third paper of this PhD thesis there are only few studies focusing on biomarkers in febrile seizures but those published show a wide variety of different biomarkers. One study showed a negative correlation between the levels of Interleukin-6 (IL-6), serum iron levels and transferrin saturation in the cases of febrile seizures in children presenting at the emergency ward. The authors discuss that if the role of IL-6 in febrile seizures is known, an anti-cytokine drug could prevent febrile seizures in high-risk patients (Gupta S). Another study showed that the low inflammatory IL-1RA (interleukin-1 receptor antagonist) to proinflammatory IL-6 ratio appears to be a possible biomarker for acute hippocampal injury in febrile status epilepticus and may have prognostic ability to differentiate children at risk of developing mesial temporal lobe epilepsy (Gallentine et al., 2017). Whereas our results show that serum neurofilament light chain is not elevated during short recovery time after febrile seizures a Turkish group interestingly demonstrated that urinary levels of kidney injury molecules were increased in patients with febrile seizures compared to age and gender matched healthy controls and therefore suggest a possible subclinical renal damage in these patients (Güneş et al., 2016). The difference may lie in the fact that the brain has a bigger ability than the kidney to recruit additional capillaries when exposed to hypoxia (Evans, Gardiner, Smith, & O'Connor, 2008; Nippert, Biesecker, & Newman, 2018). In contrast to our results the authors of a rodent study conclude that simple febrile seizures can cause neuronal damage in the hippocampal dentate gyrus which they could detect in stained sections of rats (Nazem et al., 2012). Further attention has been drawn to the role of electrolytes, in particular sodium, in febrile seizures with the theory, that low sodium levels may increase the risk for complicated febrile seizures with repeated convulsions (Hugen, Oudesluys-Murphy, & Hop, 1995; Kiviranta & Airaksinen, 1995). This hypothesis has been refuted almost a decade later where no difference in sodium levels was found comparing children with simple febrile seizures to those with recurrent seizures, whereas children with febrile seizures in general seem to have lower sodium levels than children with afebrile seizures (Thoman, Duffner, &

Shucard, 2004). A Finnish group published their findings which go in line with our results – they measured S100B concentrations in serum and CSF of children after their first febrile seizure and compared them with children with acute infections. Their data indicate that febrile seizures do not cause significant blood-brain barrier openings and therefore, as generally accepted, seem to be harmless for the developing brain (Mikkonen et al., 2012). For all physicians it is always very important to be able to differentiate serious diseases from harmless disorders, with a standard approach of analyzing NfL in children presenting with (febrile) seizures at the emergency ward, it could support in decision making but also in reassurance of frightened parents. Postictal measurement of the N-terminal fragment of BNP (NT-proBNP) also seems to help discriminate different types of epilepsy in childhood inter alia also febrile seizures (Rauchenzauner et al., 2007) just like copeptin showing higher levels in children with febrile seizures compared to febrile controls (Stöcklin et al., 2015). In conclusion there are only few empirical studies dealing with biomarkers in febrile seizures, however, there seems to be considerable interest in exploring biomarkers in general in the pediatric population and specifically NfL as being a sensitive marker of neuroaxonal injury.

Limitations and outlook

We are aware that our research may have some limitations. First, we only enrolled pregnant women at risk of developing preeclampsia, hence there is some likelihood that dissimilar evaluations would have arisen if the healthy control group had been without any risk factors. This draws attention to the fact that studies on NfL levels in healthy collectives are rare, especially in healthy pregnant women. Second, it underlines the difficulty of collecting serial data since averagely two samples per participant were collected which might be a source of uncertainty. Further experimental investigations are needed to gain more knowledge on NfL levels in different age groups and to find out which clinical characteristics per se and to what extent certain risk factors have impact on them. Unfortunately we did not have samples from later timepoints after delivery which could give additional information on the dynamics after we have demonstrated the rise in NfL after birth. We had also stated that CSF samples could help to validate the intracerebral origin of NfL, this was conducted by a Swedish research group not much longer thereafter where increased CSF concentrations pointed to a neuroaxonal injury in preeclampsia (Andersson et al., 2021).

Concerning the pediatric cohort it might be misleading that NfL levels increase with fever but also correlate with CRP levels which might suggest that fever itself may subsequently lead to elevated NfL levels. Once again it is inevitable to perform large studies on NfL, also with in order to compare different pediatric age groups and children with different underlying diseases.

Conclusion

With the advent of the single molecule array (Simoa) technology as a digital immunoassay, the sensitivity has significantly improved and allows the reliable and accurate quantification of serum NfL levels. In this PhD thesis we demonstrated that NfL can serve as a predicitive and diagnostic biomarker in different patient groups with a broad variety of underlying diseases. Further we were able to confirm that NfL is an age-dependent unspecific marker of tissue injury in the CNS (Gaetani et al., 2019). Moreover we showed for the first time that maternal serum NfL levels increase after parturition. In conclusion we state that NfL plays a growing role in the assessment of neurological impact of various clinical settings in everyday clinical practice and is a promising biomarker for use in routine management of patients with an affected CNS. Additional studies addressing the clinical utility of NfL will help standardize its use and support to understand which clinical characteristics influence NfL levels.

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Curriculum Vitae

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Personal details

Name:	Evers Katrina
Date of birth:	28.03.1984 in Perth, Australia
Nationality:	Austrian, Australian
Family status:	In a stable relationship, 3 children: Liam, born 20.05.2012; Louis, born 20.04.2014; Mavie, born 29.06.2017

Academic education

11/2015-10/2021:	Doctoral candidate, PhD program Clinical Research, University of Basel, „Novel biomarkers in perinatology“ Supervisor: Prof. Sven Wellmann
10/2007-05/2012:	Doctor of Medicine, Medical University Graz, Diploma thesis: „Does fracture instability cause leg length discrepancy?”
10/2003-10/2007:	Graduate engineer, Medical engineering, University of Applied Sciences Linz, Diploma thesis: “Development of an algorithm for semi-automatic segmentation of the bony tissue of the knee joint based on magnetic resonance images”

Professional experience

Starting in 11/2021:	Fellow – Paediatric Nephrology, University Children's Hospital Zurich
Since 10/2021:	Specialist in paediatrics, SIWF/FMH
01/2021 – 10/2021:	Resident – Paediatric Nephrology, Inselspital Bern University Hospital
11/2019 – 10/2021:	Fellow – Paediatric Nephrology, University Children’s Hospital Basel
01/2019 – 12/2019:	Deputy senior physician – Service de pédiatrie, Hôpital du Jura - Delémont (50%)
07/2018-12/2018:	Deputy senior physician – Emergency unit, University children’s hospital Basel (50%)
21.06./19.10.2018:	Theoretical written and oral practical examination in paediatrics
12/2012-06/2018:	Resident – University children’s hospital Basel, paediatric surgery and paediatrics, 50-100%
02-03/2012:	Clinical training final year – University hospital Basel, Department of internal medicine
01/2012:	Clinical training final year – Women’s Health Clinic of University Hospital Basel
11-12/2011:	Clinical training final year – University hospital Balgrist, Zürich, Department of orthopaedics
10/2011:	Clinical training final year – Practice Dr. Joachim Leisch, Linz

Scientific collaboration

01/2015-10/2021:	Fetal and Neonatal Stress Research Group, Prof. Sven Wellmann, University children's hospital Basel
06/2009-05/2012:	Research group, Prof. Annelie-Martina Weinberg, Department for paediatric surgery/Orthopaedics, Medical University Graz

Grants and awards

01/2016-12/2016:	50% salary in the course of "Special program paediatric research"
01/2007:	Dräger Best Student

Scientific contributions to conferences

Evers, KS
Novel model for individual fetal growth prediction and risk stratification
7th Congress of the European Academy of Paediatric Societies, Oct 30th-Nov 3rd, 2018, Paris; Oral presentation

Evers, KS
Novel model for individual fetal growth prediction and risk stratification
UKBB Research Day, Oct 23rd, 2018, Basel; Chair and poster presentation

Evers, KS
Impact of parturition on maternal neuronal integrity
jENS: 2nd Congress of joint European Neonatal Societies, Oct 31st - Nov 4th 2017, Venice; Poster presentation

Evers, KS
Impact of parturition on maternal neuronal integrity
UKBB Research Day, Oct 19th, 2017; Basel; Poster presentation

Evers, KS, Zutter A, Hauri K, Uhde S, Berset A, Kühne M, Donner BC
Chaotic arrhythmia during successful resuscitation after ingestion of yew (Taxus baccata) needles; Annual meeting SSP & SSAI, June 1st, 2017
St. Gallen, Poster presentation

Evers, KS
Association of axonal injury and preeclampsia; Clinical Research Day, Jan 19th, 2017; Basel, Poster presentation

Evers, KS
Association of axonal injury and preeclampsia
Annual Meeting of the Swiss Society of Neonatology, Jan 10th, 2017; Zürich, Oral presentation

Evers, KS; Wellmann, S
Fetal stress hormones at birth – a systematic analysis of cortisol, norepinephrine,

arginine vasopressin and copeptin; UKBB Research Day, Oct 27, 2016; Basel; Poster presentation

Evers, KS; Wellmann, S
Fetal stress hormones at birth – a systematic analysis of cortisol, norepinephrine, arginine vasopressin and copeptin
The 6th Congress of the European Academy of Pediatric Societies, Oct 21-25, 2016; Geneva; E-Poster Discussion Presentation

Evers, K; Widni, E; Rupp, M; Zötsch, S; Kroneis, K; Weinberg, AM
Histologische Untersuchung des Einflusses der Stabilisierung diaphysärer Frakturen auf die physäre Chondrozyten-Proliferation am Rattenmodell
47. Jahrestagung der Österreichischen Gesellschaft für Unfallchirurgie, Oct 6-8, 2011; Salzburg; Oral Presentation

Personal skills and competences

Languages:	Mother tongue:	German, English
	Others:	Spanish – good reading, writing and verbal skills French – fluent
Qualifications:	Diagnostic conversation – How do I tell parents, that their child is severely ill or disabled? Representative of laser safety according to EN 60825 part 1 Training for anaesthesia machines Matlab; GCP basic course/GCP Clinical Research involving Children	

Additional education

02/2016 - 11/2016:	Antelope – competitive career program for excellent junior female scientists:
09/2016:	3-Day-Training: How to present at international Conferences
06/2016:	Expert Exchange: Prof. Klas Blomgren, Department of Women's and Children's health, Karolinska Institute, Stockholm
05/2016:	3-Day-Training: How to publish in peer-reviewed Journals
04/2016:	2-Day-Training: Successful Self-marketing
02/2016:	1-Day-Training: How to become a more efficient Researcher

Memberships

FMH, VSAO, ESPR, SGP, GPN