ABSTRACT

Introduction Hepcidin production is normally upregulated by iron stores, and in obesity has been shown to be overexpressed and correlated with low iron status. The increased hepcidin may restrain the iron release from the cells by affecting the expression of ferroportin, which probably associates with the development of diabetes complication. First, we investigate the difference of serum hepcidin and iron parameters between obese and non-obese pregnant women; second, we examine the correlation between serum hepcidin and adverse maternal and neonatal outcomes in pregnant women.

Methods and analysis This is a mono-centre, prospective cohort study with a study (obese) and a control group (non-obese women). In the first trimester, 188 singleton pregnancies will be recruited. Thereof, we expect 75 with a body mass index (BMI) ≥30 kg/m² and 113 with a BMI 18.5–30 kg/m². Serum hepcidin, iron and haematological parameters will be measured at 11–14, 24–28, 32–36 weeks of gestation and at time of delivery. Blood pressure, weight, BMI and smoking status will be examined at all visits. We will assess the composite endpoints adverse maternal outcomes (including pre-eclampsia, gestational hypertension, gestational diabetes mellitus, haemorrhage, placenta abruption) and adverse neonatal outcomes (preterm birth, intrauterine growth restriction, preterm premature rupture of membranes, Apgar score <7 at 5 min, stillbirth, neonatal death). Recruitment has started in April 2019.

Ethics and dissemination This study received ethical approval from the ethics committee in Basel. The results of the study will be published in a peer-reviewed journal, and presented at national scientific conferences.

Trial registration number NCT03792464.

INTRODUCTION

Obesity is the most common problem in obstetrics that affects both the mother and her offspring. Obesity causes short-term and long-term problems for the mother, such as increasing her risk of gestational diabetes mellitus (GDM) and pre-eclampsia during pregnancy and further increases the risk of developing the metabolic syndrome in later life. The offspring have an increased risk of obstetric morbidity and mortality and, consistent with the developmental origins of health and disease, a long-term risk of childhood obesity and metabolic dysfunction.1

Obesity is also associated with an increased risk of iron deficiency, attributable to adiposity-related inflammatory mediators on iron regulatory pathways.2 The pro-inflammatory cytokine interleukin-6, frequently elevated in obesity, has been shown to induce expression of hepcidin,3 a negative regulator of intestinal iron absorption and macrophage iron efflux. Normal fetal growth and development is dependent on maternal iron sufficiency during pregnancy. Pregnant women have an increased requirement of iron to support feto-placental development, expansion of maternal red blood cell (RBC) mass, and to compensate for intrapartum blood loss; to meet this need, absorption of dietary iron...
is enhanced concomitantly with increased utilisation of existing iron stores. During a healthy pregnancy, hepcidin is reduced, enabling iron transfer to the fetus.3–6 Obesity in pregnancy may lead to hepcidin excess and decreased iron transfer to the fetus.7–9 Low iron stores are reported to be more common in obese pregnant women.10 The correlation between serum ferritin and hepcidin has been shown in previous studies.3,5,8,11–12 Although in other studies, no correlation between serum hepcidin and iron status has been found.4,6,13 To strengthen the link between the poor iron status of obesity and hepcidin, there is an interesting observation that hepcidin is expressed not only in the liver but also in adipose tissue and that mRNA expression is increased in adipose tissue of obese pregnant patients.7 The increased hepcidin may restrain the iron release from the cells by affecting the expression of ferroportin, which probably associates with the development of diabetes complication.14 Iron content appears to be increased in the subcutaneous and visceral adipose tissue of obese patients, and negatively correlated with adiponectin expression, which could be contributing to insulin resistance and the metabolic complications of obesity.15

There are few studies examining the influence of maternal obesity on maternal iron status, with some indicating that iron status may be compromised,7–9,10 and others reporting no impact.17 However dietary or iron supplementation was not assessed and might be responsible for divergent results. There is no study evaluating the difference of serum hepcidin and ferritin between obese and non-obese pregnant women longitudinally during pregnancy as well as the association between these parameters and adverse pregnancy outcomes.

METHODS AND ANALYSIS

Primary objective
The primary objective is serum hepcidin level (ng/mL) measured at 11–14 weeks of gestation.

Secondary objectives
1. To compare iron status parameters, haemogram and C-reactive protein (CRP) measured in the first trimester between obese and non-obese pregnant women.
2. To describe the course of serum hepcidin, iron status parameters, haemogram, CRP, weight and body mass index (BMI) during pregnancy and to compare them between obese and non-obese pregnant women.
3. To describe the correlations between serum hepcidin, iron status parameters, haemogram, CRP, weight and BMI in each trimester and at delivery.
4. To investigate whether adverse pregnancy outcome is associated with serum hepcidin and iron status in the first trimester and during total pregnancy.
5. To investigate whether adverse neonatal outcome is associated with serum hepcidin and iron status in the first trimester and during total pregnancy.

Study design
This is a mono-centre, prospective cohort study with a study (obese) and a control group (non-obese women). This study protocol was developed on the basis of Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT; see online SPIRIT checklist for further details).

Study settings
The study will be conducted at the University Hospital of Basel, Department of Obstetrics and Antenatal Care with 2800 deliveries/year. The study has started in April 2019. We estimate to recruit all patients within 18 months. A total of n=188 healthy pregnant women (expected 75%±40% with BMI ≥30 kg/m²) will be recruited in the first trimester in our outpatient department. Considering a dropout rate of 10% we will have a total of 169 evaluable women.

Eligible criteria
Inclusion criteria
► Age≥18 years.
► Singleton pregnancy.
► BMI ≥18.5 kg/m².
► Gestational age at recruitment: 11–14 of gestational weeks.
► Written informed consent.

Exclusion criteria
► Fetal genetic, chromosomal or intervention requiring morphological abnormalities.
► Chronic disease of heart, liver, kidney, cardiovascular system, gastrointestinal tract, neurological, autoimmune, haematological disorders and psychiatric disorders or known infection like hepatitis or HIV.
► The inability to read and/or understand the participant’s information sheet.

Assessment of primary outcome
The serum hepcidin will be investigated at 11–14, 24–28, 32–36 weeks of gestation and at labour. Blood samples are collected by venepuncture. The measurement of serum hepcidin will be conducted at the University Hospital of Basel, Department of Biomedicine. Serum hepcidin will be measured with an ELISA. No additional visit is necessary besides the standard routine antenatal care visits.

Assessment of secondary outcomes
Serum iron parameters (serum ferritin and soluble transferrin receptors) will be measured at 11–14, 24–28, 32–36 weeks of gestation and at labour. Iron parameters will be determined using routine laboratory methodology. Blood samples will be collected by venepuncture. All blood measurements will be conducted at the University Hospital of Basel, Department of Laboratory Medicine and Department of Biomedicine.

The following maternal outcomes will be investigated: pre-eclampsia defined according to the Report of the American College of Obstetricians and Gynecologists’
Task Force on Hypertension in pregnancy,\textsuperscript{18} pregnancy-induced hypertension,\textsuperscript{18} infection in pregnancy (urinary tract infection, vaginal infection, chorioamnionitis and so on), anaemia and iron deficiency in pregnancy according to the WHO with ferritin cut-off of 15 µg/L,\textsuperscript{19} cholestasis of pregnancy, GDM according to the results of an oral 75g glucose test, mode of delivery, abnormal placenta, placental abruption, thromboembolism in pregnancy and puerperium, peripartum and postpartum haemorrhage, estimated blood loss, anaemia in postpartum, puerperal infection or sepsis, transfusion requirement. Pre-eclampsia, gestational hypertension, GDM, cholestasis of pregnancy, peripartal haemorrhage (increased blood loss peripartal or significant haemoglobin (Hb) difference between before and after delivery >30g/L), infections, anaemia and placenta abruption are defined as adverse maternal outcomes.

The following neonatal outcomes should be investigated: gestational age at birth, birth weight, Apgar score, pH levels, preterm delivery <37 weeks of gestation, preterm premature rupture of membranes (PPROM), macrosomia with birth weight above 95th percentile, intrauterine growth restriction (IUGR) with birth weight below 5th percentile, low birth weight with birth weight below 2500g, stillbirth defined according to WHO,\textsuperscript{20} neonatal death defined according to UNICEF and WHO,\textsuperscript{21} admissions to the neonatal intensive care unit. Preterm birth, IUGR, PPROM, macrosomia, Apgar score <7 at 5, stillbirth and neonatal death are defined as adverse neonatal outcomes.

**Participant timeline and study procedures**

All healthy pregnant patients with regular care at our outpatient department are counselled and asked at 6–10 weeks of gestation to participate. At 10+0 to 13+6 weeks of gestation, all women have to undergo a first trimester ultrasound scan which is standard care. The ultrasound scan is used to confirm gestational age and to diagnose any major fetal abnormalities. All pregnant women who meet inclusion/exclusion criteria will be recruited in the first trimester in the framework of regular pregnancy visit through physicians, midwives and study coordinators. Serum hepcidin, iron and haematological parameters will be measured at 11–14, 24–28, 32–36 weeks of gestation and at labour. The blood pressure, weight, weight gain, BMI and smoking status will be examined at all visits as a standard of care. Time and event schedule provides an overview about the schedule of observations and assessments (table 1).

**Haematological parameters**

This is a routine examination on visits 1–4. Hb, RBC count, haematocrit, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb

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**Table 1** Time and event schedule

<table>
<thead>
<tr>
<th>Visits</th>
<th>Enrolment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>11–14</td>
<td>24–28</td>
<td>32–36</td>
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</table>

BMI, body mass index; CRP, C-reactive protein.
concentration (MCHC), percentage of microcytic erythrocytes, hypochromic RBC and reticulocyte Hb content are measured using a haematology analyser. MCH is automatically calculated from Hb and RBC.

**Serum ferritin, CRP, hepcidin and soluble transferrin receptors**

There are additional examinations on visits 1–4. Ferritin is assessed by chemiluminescence immunoassay and CRP is assessed by immunoturbidimetry. Soluble transferrin receptors and serum hepcidin are measured with an ELISA.

**Recording of medications**

All medications being continued by a patient on enrolment and all medications given in addition must be documented on the case report form (CRF) and in the patient’s medical records.

**Sample size**

Sample size is estimated so that a difference between obese and non-obese pregnant women regarding the primary endpoint—serum hepcidin level in the first trimester—can be shown with 80% power, at a significance level $\alpha=5\%$.

Sample size estimation is based on the following assumptions:
- The proportion of obese women is expected to be 40%.
- We assume hepcidin levels to be normally distributed with equal variance for obese and non-obese women.
- Based on the results reported in Dao *et al.* for obese women in the second trimester, and assuming these are similar in the first trimester, we assume a mean hepcidin level of 13.5 ng/mL in the first trimester and a SD of $\sigma=9.0$ ng/mL. Dao *et al.* report a mean difference of 8.0 ng/mL between obese and non-obese women, whereas Garcia-Valdes *et al.* report a mean difference of approximately 5.0 ng/mL. Here, we aim to show a difference of 4.0 ng/mL, thus we assume $\mu=17.5$ ng/mL in non-obese women. A clinical significance of the difference of 4.0 ng/mL will be monitored examining the correlation between serum hepcidin and adverse pregnancy outcomes.

Sample size was calculated using a re-sampling procedure. Each sample size ($n_i=1, \ldots, 71=20, \ldots, 300$) was evaluated, first determining the number of obese women (expected 40%, c.f. above) by sampling 999 times from a binomial distribution with $n=n_i$ and probability $p=0.4$. Then, 999 times $n_i$ observations were sampled from the above described distributions for non-obese women and obese women, respectively. The difference in each sample between obese and non-obese women was then tested using a two-sample $t$-test. The null hypothesis was rejected when the difference was statistically significant ($p<0.05$). Sample size was set to ensure a significant result in at least 80% of the cases (power: $1-\beta=0.8$), at a significance level $\alpha=5\%$.

Supposing to show a hepcidin difference of 4.0 ng/mL, a total of 188 women should be recruited in order to have a total of 169 evaluable women, considering a dropout rate of 10%. A sample size re-estimation is planned as soon as hepcidin levels of the first trimester of 80% of the initially estimated number of women (ie, 136 out of 169) will be available (c.f. interim analysis). P values resulting from secondary analyses will not be interpreted as confirmative but will be used to identify hypotheses worth of further investigation.

**Data collection**

The study data recorded in the CRF will be transferred to a corresponding electronic CRF (e-CRF) by a designated person. The data will be extracted from clinical records by study nurses and study coordinators. The principal investigator will be responsible for assuring that the data entered into the e-CRF is complete, accurate, and that the entry and updates are performed in a timely manner. All information recorded in the e-CRFs will be traceable to the source documents in the patient’s file and in the date source files.

The e-CRF will be implemented by the data management group at the Clinical Trial Unit Basel using the electronic data capture (EDC) software SecuTrial. The EDC system runs on a server maintained by the IT department of the University Hospital Basel.

Internal data management will be conducted. The data review and data-handling documents, to be developed during the initiation phase of the study, will include specifications for consistency and plausibility checks on data and will also include data handling rules for obvious data errors.

In compliance with the International Council on Harmonization (ICH) and good clinical practice (GCP) guidelines the investigator/institution will maintain all source documents that support the data collected from each patient, and all documents as specified in Essential Documents for the Conduct of a study and as specified by the applicable regulatory requirements. The investigator/institution will take measures to prevent accidental or premature destruction of these documents. Essential documents must be retained until at least 10 years after the last approval. If the responsible investigator retires, relocates or, for other reasons, withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility.

**Withdrawal and discontinuation**

Patient’s participation may be terminated prior to completing visit 4 for any of the following reasons:
- The patient withdraws her informed consent for whatever reason.
- Non cooperative patient, lack of compliance.
- Occurrence of a transient disease, which might distort the results of this study or represents a contraindication.
Premature termination of the study must be agreed on by the principal investigator and must be documented. Reasons for premature termination include inadequate recruitment of patients, a protocol violation was identified or developed during the study, failure of responsible investigator to comply with the protocol or GCP guidelines.

Planned analysis

The statistical analysis will be conducted by the Clinical Trial Unit Basel. Detailed methodology for summaries and statistical analyses of the data collected in this study will be documented in a statistical analysis plan. The statistical analysis plan will be finalised before database closure and will be under version control at the Clinical Trial Unit Basel. The Full Analysis Set will consist of all included women for whom serum hepcidin level in the first trimester (11–14 weeks of gestation) is available. Demographics and baseline characteristics will be reported for obese and non-obese women separately and taken together.

Primary analysis

The primary objective is to compare the primary endpoint between obese and non-obese pregnant women. If data are approximately normally distributed, a two-sample t-test will be applied. Otherwise, data transformation (eg, log), the use of a generalised linear model with a Poisson error distribution or the use of a non-parametric Wilcoxon two-sample rank test will be considered.

Secondary analyses

Secondary endpoints measured in the first trimester (secondary objective 1) will be compared between obese and non-obese pregnant women as described for the primary endpoint. The course of the secondary endpoints during pregnancy (secondary objective 2) will be compared between obese and non-obese pregnant women using a random slope/random intercept linear mixed effects models for each endpoint. The model will include the respective secondary endpoint as dependent variable, study group (obese vs non-obese), gestation week (continuous) and the interaction between study group and gestation week as fixed effects and women (patients ID) as random effect with random slope and random intercept. In case of an obvious non-linear time course, time will be included as categorical variable (time point: first, second and third trimester and delivery) instead, and a random intercept model will be applied (thus no random slope).

The correlations between secondary endpoints measured during the course of pregnancy (secondary objective 3) will be described by means of scatterplots and Spearman’s rank correlation coefficient for each trimester and at delivery separately. Adverse maternal neonatal outcomes will be analysed for an association with the listed secondary endpoints in the first trimester using logistic regression (secondary objectives 4 and 5).

As a first step, univariate models will be fit. Each model will include the respective binary endpoint as dependent variable, and one of the secondary endpoints measured in the first trimester as explanatory variable. In order to test for a combined effect of the secondary endpoints, in a second step, multivariable logistic regression models will be fit. A forward model selection approach will be applied, based on Akaike’s information criterion (AIC). Starting with the ‘null model’—the simplest model, including no explanatory variable—single predictor variables (ie, secondary endpoints) will repeatedly be added if and as far as AIC can be decreased. This will result in a final model that includes the set of variables that (together) best describe the outcome. (A forward model selection approach is chosen, due to the relatively large number of predictors of interest in comparison with the expected number of events.) For each statistical model, odds ratios will be presented for each explanatory variable with 95% CIs and p values. Further, the frequency of each composite will be given for obese and non-obese women.

Interim analysis

The estimation of the variance (or the distribution in general) of hepcidin levels is crucial for determining the sample size of the study, but hepcidin levels observed in the study may deviate considerably from our assumptions. We will therefore re-estimate the sample size as soon as hepcidin levels of the first trimester of 80% of the initially estimated number of women (ie, 136 out of 169) will be available. We will re-estimate the variance $\sigma^2$ obese and $\sigma^2$ non-obese in a blinded manner—only the hepcidin levels of the third trimester will be available to the statistician performing the interim analysis. The overall variance $\sigma^2$ will be estimated (one-sample variance estimator), assuming $\sigma^2 = \sigma^2$ obese = $\sigma^2$ non-obese. Since no hypothesis test is performed, no p value adjustment to control type I error is needed. Using the re-estimated SD, the sample size N’ will be re-estimated as before. If there should be clear evidence against a normal distribution of hepcidin levels (based on visual inspection of quantile–quantile plots), a t-test based on transformed data or a non-parametric Wilcoxon two-sample rank test will be used. The sample size will be increased in order to include N’ evaluable patients whenever N’>N, preserving a power of 80%. A sample size reduction or early stopping of the study will not be considered.

Deviations from the original statistical plan

If substantial deviations of the analysis as outlined in these sections are needed for whatever reason, the protocol will be amended. All deviations of the analysis from the protocol or from the detailed analysis plan will be listed and justified in a separate section of the final statistical report.

Handling of missing data

Presumably, there may be reasons for dropouts confounded with the measurements. For example, some
composites of adverse maternal and neonatal outcomes will lead to a preterm end of pregnancy, which will result in missing measurements after the first trimester. These data will be missing not at random. It is important to keep these dropouts in the analysis set regarding secondary objectives 4 and 5. In case there will be many such dropouts (ie, missing not at random), inverse probability of censoring weights will be considered, and a sensitivity analysis with a complete case analysis set will be performed.

Missing data and dropouts will be handled using the most appropriate method, based on an examination of missing values before data base closure. Details will be specified in the statistical report and analysis plan. The number of missing values in each endpoint and the number of dropouts will be summarised for obese and non-obese women.

Confidentiality and coding
Project data will be handled with uttermost discretion and is accessible only to the authorised personnel who require the data to fulfil their duties within the scope of the research project. On the CRFs and other project-specific documents, participants are identified only by a unique participant number. Biological material in this project is not identified by a participant name but by a unique participant number.

Biological material and health-related personal data will be coded. The coding key will be located by the investigator and the members of her research team. Only the investigator and the members of her research team have access to this key. Biological material is appropriately stored in a restricted area accessible only to the authorised personnel. Data generation, transmission, storage and analysis of health-related personal data and the storage of biological samples within this project will follow the current Swiss legal requirement for data protection strictly and will be performed according to the human research ordinance (HRO) Art. 5. Health-related personal data captured during this project and biological samples from participants are strictly confidential and disclosure to third parties is prohibited; coding will safeguard participants' confidentiality. Only the investigator and members of her research team will have access to project plan, dataset, statistical code and so on during and after the research project.

Retention and destruction of study data and biological material
The investigator/institution will take measures to prevent accidental or premature destruction of these documents. Essential documents must be retained until at least 10 years after the last approval. After measurement, biological material will be destroyed in University Hospital of Basel, Department of Laboratory Medicine.

Data sharing
After publication of the study, individual anonymous participant data including variable keys will be available on request to the corresponding author. Researchers may request data to repeat the analyses or use the data for secondary analyses (eg, systematic review and meta-analysis).

Patient and public involvement
Patients and the public were neither involved in developing the hypothesis, the specific aims or the research question, nor were they involved in developing plan for design or implementation of the study.

DISCUSSION
Obesity is a low-grade chronic inflammatory condition linked to the overexpression of hepcidin and secondarily to iron homeostasis. Increased hepcidin in obese pregnant women without GDM was shown in previous studies.7 9 22 23 Obesity-related inflammation may induce hepcidin biosynthesis with a consequent reduction of iron supply.7 24 Anelli et al showed lower Hb concentration in obese pregnant women.22 Interestingly, anaemia was not related to the increase in hepcidin, while this was positively associated with maternal BMI.22

Few studies have evaluated the influence of obesity on hepcidin and iron status during pregnancy, but their results are inconclusive. While increased hepcidin in obese pregnant women was shown in previous studies,7 9 22 23 it has not been confirmed in another studies.25 26 Due to previous data, we expect to observe an increase in the serum hepcidin in obese pregnant women, consequentially increased soluble transferrin receptors as a marker for iron deficiency in cells and a lower level of Hb because of iron deficiency.

It is unclear which obese pregnant women develop complications such as GDM, pregnancy-induced hypertension, pre-eclampsia, intrahepatic cholestasis, IUGR and so on during pregnancy. Increased serum hepcidin could indicate the increased risk of adverse maternal and neonatal outcomes. Chen et al showed that increased hepcidin restrains the iron release from the cells by affecting the expression of ferroportin, which probably associates with the development of diabetes complications.14 High ferritin in the first trimester was also associated with GDM.27 28

The role of hepcidin in pre-eclamptic pregnant women is unclear. Hepcidin levels in the first half of pregnancy were found significantly higher in women who subsequently developed pre-eclampsia compared with mothers having a physiological pregnancy until term.29 Reason could be due to an inflammatory condition that characterises the pre-eclamptic syndrome long before the onset of symptoms. The identification of women at elevated risk of pre-eclampsia may be sufficiently early to allow the prophylactic use of low-dose aspirin, which has been demonstrated to reduce the prevalence of pre-eclampsia when started before 16 weeks gestation.

For the first time to our knowledge, a prospective study will be performed in order to verify the potential
association between hepcidin in obese women and pregnancy outcomes such as intrahepatic cholestasis of pregnancy, haemorrhage, IUGR and so on. However, an important limitation of this study may be the size of the study population. We only powered for our primary outcome, not for secondary analyses. On the other hand, our findings could generate the first signs of the potential association between hepcidin and adverse pregnancy outcomes.

In conclusion, we will contribute new information regarding the effects of obesity on iron and hepcidin levels. Our findings will improve the understanding of hepcidin and iron metabolism in obese pregnant women and provide direction for maternal and fetal complications. Our results could initiate planning and conducting of powered studies to verify the potential association between hepcidin and adverse pregnancy outcomes. Acknowledgement of this hypothesis may conduces to establish new promising biomarkers and possible life care intervention, aimed at reducing the obesity-related risks in pregnancy.

Ethics and dissemination
All members of the research team are aware of the GCP guidelines for obtaining consent. The principal investigator must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the study. This protocol is to be followed exactly. To alter the protocol, amendments must be written, approval from the appropriate personnel and Ethics Committee approval prior to implementation should be received. Administrative changes (not affecting the patient’s benefit/risk ratio) may be made without the need for a formal amendment.

Before each patient is admitted to the study, signed informed consent will be obtained from the patient according to the regulatory and legal requirements. This consent form must be dated and retained by the investigator as part of the study records. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines.

The physicians and study coordinators must explain to potential participants the aims, methods, reasonably anticipated benefits of this study. Patients will be informed that they are free not to participate in the study and that they may withdraw consent to participate at any time. They will be told that competent authorities may examine their records and authorised persons but that personal information will be treated as strictly confidential and will not be publicly available. Patients must be given the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the patient’s dated signature.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be approved and signed by all patients subsequently enrolled in the study.

The findings of this study will be published in a peer-reviewed journal, and presented at national scientific conferences, to disseminate the results to academic and health professional audiences, and made available on our website to participants and to a wider public at the time of publication.

Contributors GAB is the principal investigator who designed the study and drafted the article. DRV is in charge with the statistical analysis. H reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of Basel (Protocol ID: 2017-02322).

Provenance and peer review Not commissioned; externally peer reviewed.

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