RESEARCH ARTICLE

REVISED Cohort profile: Improved Pregnancy Outcomes via Early Detection (IMPROvED), an International Multicentre Prospective Cohort [version 3; peer review: 2 approved]

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Abstract

Background

Improved Pregnancy Outcomes via Early Detection (IMPROvED) is a multi-centre, European phase IIa clinical study. The primary aim of IMPROvED is to enable the assessment and refinement of innovative prototype preeclampsia risk assessment tests based on emerging

biomarker technologies. Here we describe IMPROvED's profile and invite researchers to collaborate.

Methods

A total of 4,038 low-risk nulliparous singleton pregnancies were recruited from maternity units in Ireland (N=1,501), United Kingdom (N=1,108), The Netherlands (N=810), and Sweden (N=619) between November 2013 to August 2017. Participants were interviewed by a research midwife at ~11 weeks (optional visit), ~15 weeks, ~20 weeks, ~34 weeks' gestation (optional visit), and postpartum (within 72-hours following delivery).

Findings to date

Clinical data included information on maternal sociodemographic, medical history, and lifestyle factors collected at ~15 weeks' gestation, and maternal measurements, collected at each study visit. Biobank samples included blood, urine, and hair collected at each study visit throughout pregnancy in all units plus umbilical cord/blood samples collected at birth in Ireland and Sweden. A total of 74.0% (N=2,922) had an uncomplicated pregnancy, 3.1% (N=122) developed preeclampsia, 3.6% (N=143) had a spontaneous preterm birth, and 10.5% (N=416) had a small for gestational age baby. We evaluated a panel of metabolite biomarkers and a panel of protein biomarkers at 15 weeks and 20 weeks' gestation for preeclampsia risk assessment. Their translation into tests with clinical application, as conducted by commercial entities, was hampered by technical issues and changes in test requirements. Work on the panel of proteins was abandoned, while work on the use of metabolite biomarkers for preeclampsia risk assessment is ongoing.

Future plans

In accordance with the original goals of the IMPROvED study, the data and biobank are now available for international collaboration to conduct high quality research into the cause and prevention of adverse pregnancy outcomes.

Keywords
Cohort profile, preeclampsia, biobank, clinical data

This article is included in the Maternal and Child Health collection.

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> **REVISED** **Amendments from Version 2**
>
> We have now updated reference one in our manuscript to ISSHP's latest hypertensive disorders of pregnancy classification, diagnosis and management recommendations for international practice.
>
> **Any further responses from the reviewers can be found at the end of the article**

Introduction

Preeclampsia is one of the leading causes of maternal morbidity and mortality in Europe[1]. It is defined as gestational hypertension (systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg (Korotkoff V)) on at least two occasions 4 hours apart after 20 weeks' gestation, but before the onset of labour, or postpartum systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg on at least two occasions 4 hours apart with proteinuria (≥300 mg/24 hours, or spot urine protein:creatinine ratio ≥30 mg/mmol creatinine, or urine dipstick protein >/= ++)[2]. Preeclampsia affects up to 5% of all pregnancies and can lead to acute problems in the liver, kidneys, brain, and the clotting system, and is associated with an increased risk of cardiovascular and metabolic diseases later in life[2–5].

Improved Pregnancy Outcomes via Early Detection (IMPROvED) is a multi-centre, European phase IIa clinical study (ClinicalTrials.gov Identifier: NCT01891240). The IMPROvED Consortium was set up to develop a clinically useful screening test for preeclampsia to assist in offering targeted surveillance or preventative strategies. To achieve this, a high calibre pregnancy biobank augmented with well-curated patient and clinical information was required to evaluate panels of metabolomic and proteomic biomarkers, which were previously shown to be predictive of preeclampsia[6,7]. In accordance with the objectives of the IMPROvED project[2], a prospective study was set up by the clinical collaborators within the IMPROvED Consortium. First-time mothers across participating maternity units in Republic of Ireland, United Kingdom, The Netherlands, and Sweden were invited early in pregnancy to participate in the IMPROvED study and to consent to the taking, and biobanking, of biospecimens at defined times during their pregnancy for analysis of (preeclampsia) biomarkers. Detailed demographic and clinical data were collected from study participants in each participating maternity unit, and maternal measurements were performed at multiple time points across their pregnancies. Furthermore, to maximise the utility of the IMPROvED cohort, detailed data on pregnancy outcomes, including key outcomes of interest such as spontaneous preterm birth (*i.e.*, delivery <37+0 weeks' gestation) and small for gestational age (SGA) (*i.e.*, birthweight <10th customised centile) were collected[2].

In parallel to recruitment taking place, the IMPROvED project foresaw for translational research to be conducted at the commercial partners. The planned research primarily focused on replacing the biomarker measurement technology as used in identifying the respective metabolite-, and protein-biomarker panels with (commercially viable) biomarker measurement technology suitable for application in clinical laboratories. The envisioned biomarker tests were labelled MetTest and ProTest, respectively[2].

The aim of this cohort profile is to firstly provide a detailed description of the IMPROvED cohort, including data collection and follow-up procedures; secondly an update on findings reported thus far by the IMPROvED Consortium regarding the goals and objectives of the IMPROvED project, and thirdly details on how the scientific community can access IMPROvED data for research projects.

Methods

Cohort setting, location, and key dates

The IMPROvED cohort contains hospital-based maternity data from Republic of Ireland (University College Cork), United Kingdom (Keele University, University of Liverpool, and University Centre Shrewsbury), The Netherlands (Erasmus MC, University Medical Center, Rotterdam), and Sweden (Karolinska Institutet, Stockholm). Recruitment took place between 29th November 2013 and 3rd August 2017. While work on the use of metabolite biomarkers for preeclampsia risk assessment is ongoing, the data and biobank are now available for international collaboration to conduct high quality research into the cause and prevention of adverse pregnancy outcomes.

All centres obtained ethical approval for the IMPROvED consortium project from their respective ethic committees (Clinical Research Ethics Committee of the Cork Teaching Hospitals: ECM5(3)06/08/13 in August 2013; West Midlands - Solihull Research Ethics Committee: 13/WM/0268 in July 2013; Medical Ethics Committee Erasmus MC of Rotterdam: NL44426.078.13 in July 2013, and EPN – Stockholm Regional Ethics Review Board: 2013-306-31-2 in April 2013). Informed consent was signed by participants at the first study visit (11+0 to 13+6 weeks' gestation).

Eligibility criteria and inclusion

Eligibility criteria included females aged 16 years or older, nulliparous, singleton pregnancy, and signed informed consent. Full exclusion criteria have been published previously[2] and included the following: unsure of last menstrual period and unwilling to have ultrasound scan at ≤20 weeks' gestation; ≥3 miscarriages; ≥3 terminations; known or suspected major foetal anomaly/abnormal karyotype; essential hypertension treated pre-pregnancy; moderate-severe hypertension at booking (BP >160/100 mmHg); diabetes mellitus; renal disease; systemic lupus erythematosus; anti-phospholipid syndrome; sickle cell disease; HIV positive; major uterine anomaly; cervical suture *in situ*; knife cone biopsy; long-term glucocorticosteroids; treatment with low-dose aspirin; or treatment with heparin/low molecular weight heparin[2].

Initially recruitment was planned in five European countries with the following recruitment targets: Republic of Ireland (N=1,000), United Kingdom (N=1,500), The Netherlands N=1,000, Sweden (N=750), and Germany (N=750)[2]. However,

the study was not feasible at the German site, and they subsequently withdrew from the study at an early stage[8]. The following samples were enrolled in each remaining countries: Republic of Ireland N=1,501; United Kingdom N=1,108; The Netherlands N=810, and Sweden N=619 (Figure 1 and Table 1).

Data collection and follow-up

Only those who consented to sampling procedures at the second (~15 weeks' gestation) and third (~20 weeks' gestation) time-points were eligible for recruitment. While participation at the first (~11 weeks' gestation) and fourth (~34 weeks' gestation) time-points were desirable, these were not mandatory. Informed written consent was obtained from all participants at their first study visit. Participants were interviewed by a research midwife at each sampling time-point and all data, including data on storage details of specimens, were entered directly into the IMPROvED database. Participants were instructed to contact the research midwife if delivery occurred before the final study visit or if they developed preeclampsia, had a spontaneous preterm birth or delivered a small for gestational age (SGA) baby[2].

First sampling (optional visit): The first study visit took place at 11+0 to 13+6 weeks' gestation. Maternal measurements were performed for height, weight, blood pressure, pulse, urinary protein, and blood glucose for a maximum of 1,076 participants. Specimens including non-fasting 30 ml blood, 10 ml mid-stream sample of urine, and sample of hair were also collected for 1,076 participants.

Second sampling: The second study visit took place at 14+0 to 16+6 weeks' gestation, resulting in a total sample size of 4,038 participants (including the 1,076 participants from the first non-mandatory study visit). If maternal measurements were not taken at the first study visit, these were taken at the second study visit. Specimens including non-fasting 30 ml blood (n=3,992), 10 ml mid-stream sample of urine (n=3,992), and sample of hair (n=3,081) were also collected. Information on demographics, current pregnancy details, and lifestyle factors were collected during this visit through interview with a research midwife. Demographic information included maternal age, marital status, ethnicity, country of birth, education, occupation, living situation, household income and type of maternity care. Current pregnancy details included information such as gravidity and history of pregnancy complications, for example, infertility, hypertensive disorders of pregnancy and stillbirth, as well as any medical conditions. Lifestyle factors included data on smoking, alcohol use, as well as multivitamin use during pre-pregnancy, during the first trimester, and by the first study visit (Table 2).

Third sampling: The third study visit took place at 19+0 to 21+6 weeks' gestation. Maternal measurements were performed for weight, blood pressure, pulse, urinary protein, and blood glucose for a maximum of 3,794 participants. Specimens including non-fasting 30 ml blood (n=3,794), 10 ml mid-stream sample of urine (n=3,794), and sample of hair (n=3,083) were also collected

Fourth sampling (optional visit): The fourth study visit took place at 32+0 to 34+6 weeks' gestation. Maternal measurements were performed for weight, blood pressure, pulse, urinary protein, and blood glucose for a maximum 1,313 participants. Specimens including non-fasting 30 ml blood (n=1,313), 10 ml mid-stream sample of urine (n=1,313), and sample of hair (n=1,024) were also collected.

At birth (optional visit): Placental samples (n=59) were taken shortly after delivery. At centres in Republic of Ireland and Sweden, blood from the umbilical cord and a sample of the cord itself were taken for 974 and 41 participants, respectively.
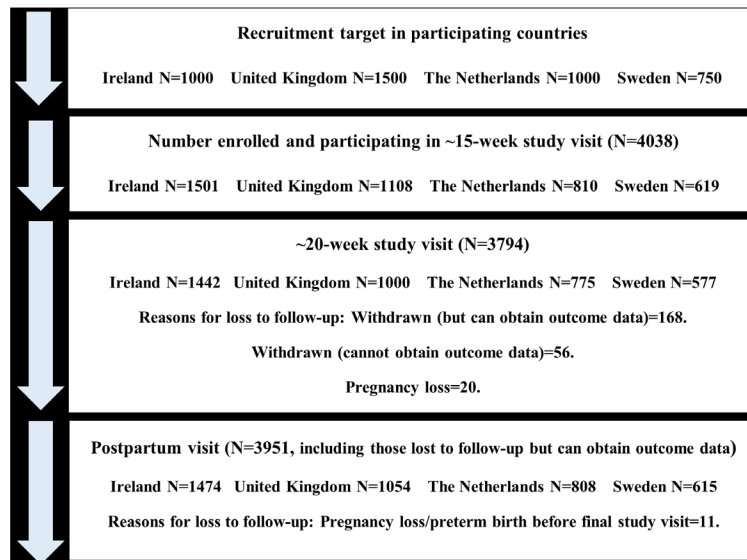

Figure 1. Flow diagram of study participants for main study visits in the IMPROvED cohort.

Table 1. Baseline characteristics of study participants enrolled in the IMPROvED cohort by country (N=4,038).

Characteristics	Total N=4038	Republic of Ireland N=1501	United Kingdom N=1108	The Netherlands N=810	Sweden N=619
Maternal age					
18–27 years	1214 (30.0)	324 (21.6)	498 (45.0)	216 (26.7)	176 (28.4)
28–37 years	2692 (66.7)	1123 (74.8)	591 (53.3)	557 (68.7)	421 (68.0)
38–47 years	132 (3.3)	54 (3.6)	19 (1.7)	37 (4.6)	22 (3.6)
Maternal body mass index					
Underweight/normal weight	2377 (59.0)	837 (55.8)	575 (52.0)	547 (67.7)	418 (67.6)
Overweight	1175 (29.1)	485 (32.4)	349 (31.5)	184 (22.8)	157 (25.4)
Obese	480 (11.9)	177 (11.8)	183 (16.5)	77 (9.5)	43 (7.0)
Missing data	6	2	1	2	1
Education					
Third level	2457 (60.9)	1056 (70.4)	573 (51.7)	371 (45.8)	457 (73.8)
Less than third level	1581 (39.1)	445 (29.6)	535 (48.3)	439 (54.2)	162 (26.2)
Employment status					
In paid employment	3609 (89.4)	1341 (89.3)	992 (89.5)	709 (87.5)	567 (91.6)
Not in paid employment	429 (10.6)	160 (10.7)	116 (10.5)	101 (12.5)	52 (8.4)
Relationship status					
Married/stable relationship	3823 (94.7)	1400 (93.3)	1044 (94.2)	775 (95.7)	604 (97.6)
Single/separated/divorced	215 (5.3)	101 (6.7)	64 (5.8)	35 (4.3)	15 (2.4)
Maternal smoking before/during pregnancy					
Non-smoker	3139 (77.8)	1093 (72.8)	882 (79.6)	644 (79.5)	520 (84.0)
Quit before first study visit	650 (16.1)	292 (19.5)	130 (11.7)	137 (16.9)	91 (14.7)
Smoked at time of first study visit	248 (6.1)	116 (7.7)	95 (8.7)	29 (3.6)	<10
Missing data	1	0	1	0	0
Maternal alcohol consumption before/during pregnancy					
Non-drinker	968 (24.0)	133 (8.9)	433 (39.1)	279 (34.4)	123 (19.9)
Quit before first study visit	3008 (74.5)	1330 (88.6)	657 (59.3)	528 (65.2)	493 (79.6)
Drank alcohol at time of first study visit	61 (1.5)	38 (2.5)	17 (1.5)	<10	<10
Missing data	1	0	1	0	0

N (%) for categorical variables.

Table 2. Prenatal and postpartum data available for IMPROvED participants.

	Time-points of Sampling Procedures					
Biobank data	*~11 weeks*	*~15 weeks*	*~20 weeks*	*~34 weeks*	*At birth*	*Postpartum (within 72 hours)*
Non-fasting 30ml blood	✓	✓	✓	✓		
10ml mid-stream sample of urine	✓	✓	✓	✓		
Sample of hair	✓	✓	✓	✓		
Umbilical cord blood[a]					✓	
Umbilical cord sample[a]					✓	
Placental samples					✓	
Maternal measurements						
Height[b]	✓	✓				
Weight[b]	✓	✓	✓	✓		
Blood pressure[b]	✓	✓	✓	✓		
Pulse[b]	✓	✓	✓	✓		
Urinary protein[b]	✓	✓	✓	✓		
Blood glucose[b]	✓	✓	✓	✓		✓
Demographics and lifestyle factors						
Maternal age	✓					
Marital status		✓				
Ethnicity		✓				
Country of birth		✓				
Education		✓				
Occupation		✓				
Living situation		✓				
Household income		✓				
Type of maternity care		✓				
Smoking		✓				
Alcohol/Drug use		✓				
Multivitamin use		✓				
Pregnancy, delivery, and infant data						
History of pregnancy complications		✓				
Medical conditions		✓				
Gravidity		✓				
Mode of delivery						✓
Infant sex						✓
Preeclampsia						✓
Gestational hypertension						✓

Time-points of Sampling Procedures						
Biobank data	*~11 weeks*	*~15 weeks*	*~20 weeks*	*~34 weeks*	*At birth*	*Postpartum (within 72 hours)*
Placental abruption						✓
Rupture of membranes						✓
Birthweight						✓
Gestational age						✓
Baby's length						✓
Baby's head circumference						✓
Estimated blood loss						✓
Apgar scores						✓
Severe neonatal morbidity						✓
Admitted to neonatal unit						✓
Reason for neonatal unit admission						✓
Paternal data						
Age[c]	✓	✓	✓	✓	✓	✓
Blood sample for DNA analysis[c]	✓	✓	✓	✓	✓	✓

[a]Taken at centres in Republic of Ireland and Sweden, only.

[b]If measurements were not taken at the first study visit, these were taken at the second study visit.

[c]Taken at centre in Republic of Ireland only, and at any one visit or by extra appointment.

Postpartum: Within 72 hours following delivery, information about the pregnancy, delivery and the baby were obtained by a research midwife through interview/reviewing medical records. The total sample size at this visit was 3,951 participants (including those lost to follow-up but for whom outcome data could be obtained from medical records, N=168). This data included, but was not limited to, information on mode of delivery, infant sex, hypertensive disorders of pregnancy, placental abruption, rupture of membranes, birthweight and gestational age of baby, Apgar scores at 1 and 5 minutes, any severe neonatal morbidity, whether infant was admitted to neonatal unit as well as reason for neonatal unit admission (Table 2). If possible, the baby's measurements were also taken at this time. If not, these were obtained from medical records. All information was confirmed by reviewing medical records. Information about complications of pregnancy, including the primary outcomes were also recorded. Any participant who developed preeclampsia, experienced spontaneous preterm birth, or delivered an SGA baby had detailed clinical, laboratory, and outcome data collected[2].

Database and biobank development
An IMPROvED customised clinical data and biobank management database was developed in Sweden. This database was specifically designed for data management in clinical trials and cohort studies. Comprehensive clinical data, blood, urine, and hair samples were collected and recorded in this database at each study visit.

In addition, IMPROvED established a high calibre pregnancy biobank containing samples from participants at each study visit. The IMPROvED pregnancy biobank is housed at University of Cork, Ireland. Both the epidemiological data and biobank samples can be used by the scientific community to conduct high quality research into maternal and child health.

Data analysis
All descriptive statistics for the current study were performed using Stata MP 14.2 (RRID:SCR_012763) (free alternative, RStudio).

Results
Baseline characteristics
Baseline characteristics of study participants are outlined in Table 1. Briefly, most participants were in the 28–37 years age bracket (66.7% overall). The United Kingdom had a higher proportion (45.0%) of younger participants enrolled (*i.e.*, 18 to 27 year-olds) compared to other countries (30.0% overall). A slightly higher proportion of obese participants were enrolled in the United Kingdom (16.5%), while a lower proportion were enrolled in Sweden (7.0%), compared to 11.9% overall. The majority of participants had a third level of

education (60.9% overall). However, this was less pronounced in the United Kingdom (51.7%) and The Netherlands (45.8%). Most participants were in paid employment across all countries (89.4% overall). Similarly, most participants were married or in a stable relationship (94.7% overall). The majority of participants were non-smokers (77.8% overall). However, a slightly higher proportion of participants smoked at time of first study visit in Republic of Ireland (7.7%) and the United Kingdom (8.7%) compared to 6.1% overall. There were fewer non-drinkers of alcohol (defined as zero alcohol intake in the three months prior to pregnancy) enrolled in Republic of Ireland (8.9%) and Sweden (19.9%) compared to the United Kingdom (39.1%) and The Netherlands (34.4%). Overall, 74.5% stopped drinking alcohol before the first visit, while 2.5% of participants in the Republic of Ireland were still drinking alcohol at time of first study visit compared to 1.5% overall. Missing data was <1% at baseline.

Findings to date

Data on 87 participants were lost to follow-up between enrolment and the final study visit (within 72 hours following delivery), resulting in 3,951 participants with postpartum outcome data. Of these, 74.0% (N=2922) had an uncomplicated pregnancy. Similar to estimates reported elsewhere[5,9], approximately 3.0% (N=122) of participants developed the primary study outcome of preeclampsia. This is subdivided by country as follows: Republic of Ireland N=57 (3.9%); United Kingdom N=30 (2.9%); The Netherlands N=24 (3.0%); and Sweden N=11 (1.8%). Other outcomes of interest including spontaneous preterm birth occurred in 3.6% (N=143) and SGA in 10.5% (N=416). Missing data was minimal (<1%) for key study outcomes, while there was ≥1% missing data for mode of delivery (1.0%) and Apgar score at 1 minute and 5 minutes (1.6% and 1.8%, respectively) (Table 3).

Discussion

Conducted analyses

ProTest: The original protein biomarker study identified, verified, and validated novel panels of protein biomarkers for the prediction of preeclampsia at ~20 weeks' gestation[7]. From this study, a panel of five protein biomarkers was selected for development into a clinical assay. These proteins were: insulin-like growth factor acid labile subunit (IGFALS), serine peptidase inhibitor Kunitz type 1 (SPINT1), melanoma cell adhesion molecule (MCAM), and the angiogenic factors placental growth factor (PlGF) and soluble endoglin (sENG), by now well-established markers for preeclampsia[10]. Throughout the protein biomarker study, various mass spectrometric techniques were applied to firstly identify[11] and then quantify the proteins of interest in a targeted fashion[12,13]. However, in 2012, mass spectrometry based multiplex protein analyses were not well established in clinical laboratory routine. A technology transfer from mass spectrometry based analyses to another multiplexing technology[14] compatible with antibody based immunoassay technologies for protein analyses was therefore deemed strategic for any future market acceptance of ProTest. As part of this transfer, specific antibody pairs for the proteins in the ProTest panel needed to be generated and evaluated for technical feasibility. With PlGF technology already available

Table 3. Number of outcomes in the IMPROvED cohort within 72 hours following delivery (N=3,951).

Outcome	N (%)
Preeclampsia	122 (3.1)
Gestational hypertension	179 (4.5)
PPROM	46 (1.2)
Placental abruption	14 (0.4)
Spontaneous preterm birth before 37 weeks' gestation	143 (3.6)
Spontaneous preterm birth before 34 weeks' gestation	34 (0.9)
Spontaneous preterm birth before 28 weeks' gestation	9 (0.2)
SGA (birthweight <10th customised centile)	416 (10.5)
Mode of delivery	
Spontaneous vaginal delivery	1973 (49.9)
Operative vaginal delivery	1003 (25.4)
Prelabour caesarean section	361 (9.1)
Emergency caesarean section	577 (14.6)
Missing	37 (1.0)
Apgar score 1 minute	
7–10 (high)	3544 (89.7)
0–6 (low/intermediate)	342 (8.7)
Missing	65 (1.6)
Apgar score 5 minutes	
7–10 (high)	3795 (96.0)
0–6 (low/intermediate)	87 (2.2)
Missing	69 (1.8)
Any severe neonatal morbidity/mortality	32 (0.8)
Infant admitted to neonatal unit	404 (10.2)

Abbreviations: PPROM, preterm premature rupture of membranes; SGA, small for gestational age. If missing data ≥1%, n (%) reported for missing data.

in the market, efforts were focused on developing a multiplex protein assay for the four other protein biomarkers. Unfortunately, severe technical issues were encountered. First, the IGFALS detection antibody cross reacted with capture antibodies against MCAM, SPINT1, and sENG, resulting in false positive results. Despite extensive further assay optimization efforts to resolve antibody cross-reactivity, the IGFALS assay could not be incorporated in a multiplex assay; a decision not to progress *de novo* antibody development for IGFALS was taken. Second, inadequate analytical sensitivity was obtained for SPINT1. Although dedicated assay parameter optimisation yielded

sufficient analytical sensitivity, these parameters were not compatible with the parameters applicable for other protein assays, rendering SPINT analysis incompatible with multiplexing techniques. By that time, the clinical use case for a 20 weeks preeclampsia screening solution was put into question by meta-analyses indicating that aspirin prophylaxis to prevent (preterm) preeclampsia needed to start before <16 weeks' gestation[15,16], this finding was corroborated by the results of the ASPRE trial[17]. Confronted with the multitude of technical hurdles and changes in screening test requirements, MyCartis (Belgium) developing ProTest abandoned the project.

Ongoing analyses

MetTest: The original metabolite biomarker study used a single LC-MS experimental set-up to analyse deproteinised metabolite extracts from patient blood specimens (EDTA plasma) collected at ~15 weeks' gestation in a discovery/validation metabolism profiling study. This resulted in a multivariate predictive model combining 14 putatively identified metabolites[6]. In contrast to proteins, mass spectrometry based multiplex metabolite analyses were well established in clinical laboratory settings at the start of IMPROvED project; notably newborn screening for inborn errors of metabolism has a worldwide clinical application[18,19]. Hence, there was no commercial imperative to migrate the metabolite analyses to another analytical technology platform. Instead, the IMPROvED translational research planning for MetTest focused on converting the results of the metabolism profiling study into targeted LC-MS analyses based on the use of reference materials, as required for future application in clinical laboratories. Early in the IMPROvED project it was found that for many of the metabolite biomarkers in the original MetTest multivariate model no reference materials were readily available, thus the original metabolite panel was not amenable to further clinical and commercial development. In response, Metabolomic Diagnostics (Ireland) developing MetTest was compelled to establish an in-house LC-MS translational research workflow centring on multiplexing targeted LC-MS assays for 10s of putative metabolite biomarkers, whereby the availability of reference materials was a selection criterion for biomarker inclusion[20].

In parallel, MetTest researchers looked into formalising screening targets for preeclampsia screening in low-risk nulliparous women. This led to novel methodology to assess predictive values, statistics relevant to clinical practitioners, directly from receiver operating characteristic curves, used by test developers to summarise a test's diagnostic performances in function of test sensitivity and test specificity[21]. Using this methodology, it was proposed that a preeclampsia risk stratification test for nulliparous should ideally mimic the preeclampsia risk information as available for a second-time pregnant woman[21]. The IMPROvED Consortium used these screening targets to perform an early cost-effectiveness analysis to assess both costs and health outcomes of a new screening test that would deliver such risk stratification[22].

During the lifetime of the IMPROvED project, PlGF gained wide-spread acceptance as an important preeclampsia risk biomarker, and early pregnancy preterm preeclampsia risk screening evolved into a separate clinical application. In response, MetTest was re-envisioned as a test that combined PlGF and metabolite biomarkers to deliver improved preterm preeclampsia as well as preeclampsia screening in nulliparous women. Using the purpose-developed LC-MS translational research workflow, candidate metabolite biomarkers were analysed in a case-control study. Models were evaluated in function of two pre-defined clinical use scenarios: (1) identify women at risk of developing preterm preeclampsia and (2) identify women at risk of developing preeclampsia at any stage of the pregnancy. It was found that combining dilinoleoyl-glycerol with PlGF effectively predicted increased preterm preeclampsia risk at ca. 15 weeks' gestation. The further addition of heptadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine expanded the capacity to also identify pregnant women at decreased risk of developing any form of preeclampsia[20].

In a further evolution of MetTest, it was shown that metabolite biomarkers can differentially predict preterm preeclampsia across body mass index classes[23], supporting the existence of distinct maternal risk profiles, a contemporary understanding in preeclampsia research[24–26]. Using machine learning methodology, these findings led to the development of novel prediction algorithms for preterm preeclampsia prediction in all pregnant women. Metabolite biomarkers augmented the established biomarkers PlGF, mean arterial pressure (MAP), and uterine artery pulsatility index (UTA-PI). Three novel prediction models were developed for three scenarios reflecting different levels of screening resources available; in each scenario use of metabolite biomarkers improved preterm preeclampsia prediction over the comparator models without metabolites. Classification of the pregnant women according to the maternal characteristics body mass index and/or race proved instrumental in achieving improved prediction[27]. The latest iteration of MetTest is currently being developed into a clinical test.

Publications

A recent publication[28] by members of the current study used IMPROvED data to examine the association between socio-economic status and pregnancy and neonatal outcomes. We did not find strong evidence of associations between individual-level socioeconomic factors and pregnancy and neonatal outcomes overall, with only few significant associations observed among pregnancy outcomes. It is anticipated that IMPROvED data will be used in further maternal and child health secondary analysis research in the future.

Other publications arising as part of the IMPROvED Consortium include a systematic review and meta-analysis examining early pregnancy biomarkers in preeclampsia[29], as well as a cost-effectiveness analysis of screening for preeclampsia in nulliparous women[22].

Strengths and limitations and further details

The IMPROvED Study has some limitations that should be noted. First, at time of enrolment, the majority of participants recruited had a third level of education, were in paid employment, and were married or in a stable relationship. Therefore, those with lower-level socioeconomic indicators may be underrepresented in the current cohort. Second, while recruitment was initially planned in five European countries (*i.e.*, Republic

of Ireland, United Kingdom, The Netherlands, Sweden, and Germany), the German site withdrew from the study at an early stage and were therefore not included in the IMPROvED cohort[8]. Third, target recruitment numbers were not met resulting in 212 fewer participants than what was originally anticipated among participating countries. However, there was little data lost (~2%) between enrolment (N=4038) and the final study visit (N=3951) reducing the potential for selection bias driven by attrition from the cohort. Fourth, participants were not universally screened for the presence of gestational diabetes mellitus (GDM), potentially underestimating the incidence of GDM in the study. Finally, data on dietary behaviours was not collected. Poor dietary patterns, before and during pregnancy, have been shown to increase the likelihood of preeclampsia and may be an important risk factor to consider in future research[30].

There are also several strengths. First, IMPROvED contains both epidemiological data and biobank data that utilised numerous aliquots on multiple media enabling high quality research into the cause and prevention of adverse pregnancy outcomes. Specimens were collected at multiple time points throughout pregnancy to enhance the range of future research that can be conducted using the IMPROvED residual biobank. Second, all data were collected in a standardised manner by trained research midwives following detailed study specific standard operating procedures. This was to ensure standardised processes across recruitment centres to minimise bias during recruitment and data collection. Third, there was minimal missing data (<1%) among baseline characteristics and key outcomes, therefore maintaining the statistical power and representativeness of the cohort[31]. Finally, this cohort profile increases awareness among the scientific community of the potential to access IMPROvED data and biobank samples. This, in turn, could foster collaborations and encourage researchers to obtain funding and ethical approval for evidence-based studies they would not normally be in a position to perform because of lack of access to large patient cohorts.

Collaboration

The IMPROvED team encourages the use of the IMPROvED cohort data for research purposes. In supplying data, the IMPROvED team must comply with its obligations of confidentiality under the Data Protection Acts of 1988 and 2000 as well as with the General Data Protection Regulation (GDPR) of 2018. The use of the data by the applicant must also be consistent with these Acts and Regulations. Therefore, only requests for anonymised data will be considered. Data sharing on a public repository is prohibited.

Data availability

Underlying data

Researchers can apply to access IMPROvED epidemiological and biobank data by contacting IMPROvED Principal Investigator, Dr Fergus McCarthy, University College Cork, Ireland in the first instance (fergus.mccarthy@ucc.ie), followed by submission of a proposal to the IMPROvED Consortium. The data are not publicly available due to privacy/ethical restrictions and only available upon reasonable request. For further information, please email Dr Gillian Maher at gillian.maher@ucc.ie.

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Open Peer Review

Current Peer Review Status: ✓ ✓

Version 2

Reviewer Report 20 June 2024

https://doi.org/10.21956/hrbopenres.15203.r39337

✓

Shaun Brennecke 🆔

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This report summarises the history, aims, content and uses-to-date of the IMPROVeD cohort, a collection of clinical data and measurements, together with various biobanked samples, from 4000 or so pregnant women.

The cohort was established mostly with a view to enabling the development of biomarkers for the prediction of preeclampsia.

This goal seems to have met with mixed success, and now the consortium of researchers responsible for the cohort is altruistically offering it for others to potentially take advantage of its rich store of data and samples.

The report itself is therefore descriptive rather than analytic in nature, and amounts to an extended and detailed invitation to potential research collaborators to consider using this large and valuable cohort to progress knowledge relating to the aetiology, prevention and improved management of pregnancy complications.

As such, the usual criteria for judging the scientific merits of an academic journal submission describing the results of a clinical study or laboratory investigation are not especially applicable to this article.

Depending on their particular needs, external researchers interested in accepting the invitation to collaborate will need to make their own individualised assessments of the potential utility of the cohort's available data and samples.

A principle of *caveat emptor* therefore applies in situations such as this, with notice being taken of the relevance or otherwise of the patient inclusion and exclusion criteria, stages of pregnancy at which data and samples were obtained, the type of data and samples that were collected, the standard operating procedures and level of quality control for sample collection, processing, aliquotting, and storage, and so on.

For example, the range of countries contributing to the cohort suggests the ethnicity/racial background of the women included in it is likely to be mostly northern European. Analyses of the

cohort may therefore not be as informative when applied to other, different ethnicities/racial backgrounds.

As well, the cohort's relatively narrow inclusion and rather broad exclusion criteria mean extrapolation of cohort analyses to other real-world pregnancy situations (multiparous women, multiple pregnancies, women with relevant comorbidities such as chronic hypertension, diabetes, SLE, CKD, APL syndrome, etc) is likely to be limited.

That said, as has been the case with other similarly available large collections, subsequent, post hoc, retrospective interrogations by external collaborators can prove of value in various ways, including by providing suggestive and/or preliminary data for further more definitive prospective studies.

Of particular benefit within the IMPROVeD cohort are the three sampling points at previable stages of pregnancy (late first/early-to-mid second timester). They provide ample scope for the determination of predictive algorithms and tests at a sufficiently early moment in a pregnancy to be not only of likely clinical value for prediction and institution of prophylactic interventions, but also of practical utility with respect to patient availability, given most pregnant women in countries with similar maternity care environments to the those providing the cohort will have attended for care by then.

This benefit is enhanced by the impressivley large size of the IMPROVeD cohort.

In terms of the report itself, as a description of the IMPROVeD cohort and as an invitation to potential collaborators to consider availing themselves of its wealth of data and samples, it meets these aims well enough.

Notwithstanding the above comments, the only suggestions I offer for changing the manuscript are to update reference 1 to the latest ISSHP pronouncement on the classification etc of the hypertensive disorders of pregnancy (see PMID: 35066406) and to correct the presumed error in Table 2 in the Biobank section wherein placental samples are purported to have been taken at 11 and 15 weeks' gestation, rather than at birth/shortly after delivery (as stated in the main body of the article on page 5).

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Preeclampsia and other complications of human pregnancy.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 04 March 2024

https://doi.org/10.21956/hrbopenres.15203.r38190

✔️ **Hongmei Li** 🆔
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Authors have answered my questions, and I have no other comments.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: hypertensive disorders in pregnancy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 30 January 2024

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? **Hongmei Li** (iD)

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This article provided a description for the profile including existing research results of IMPROvED cohort study. Overall, the cohort study was well designed. It is expected that this cohort study will have exciting results in the risk assessment for preeclampsia.

However, I have some questions:
1. First, for this research design, what is the reason for a short time interval between the first collection of specimens at 9-13 gestational weeks and the second collection at 14-16 gestational weeks ? Of the 4038 participants, only 1076 completed the first sample collection. What was the impact on the overall study?

2. Second, the dietary pattern is also a contributing factor to preeclampsia, and the dietary habits vary among different countries. Whether dietary behaviors were considered for collection when designing this cohort study? If not, it is recommended to discuss in the limitation section.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: hypertensive disorders in pregnancy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Feb 2024
Gillian Maher

Dear Dr Hongmei Li,

We thank you for your helpful review of our manuscript entitled *"Cohort profile: Improved Pregnancy Outcomes via Early Detection (IMPROvED), an International Multicentre Prospective Cohort"*.
Please find below an itemized reply addressing each comment in your Reviewer's Report.

*1. **This article provided a description for the profile including existing research results of IMPROvED cohort study. Overall, the cohort study was well designed. It is expected that this cohort study will have exciting results in the risk assessment for preeclampsia. However, I have some questions:***
First, for this research design, what is the reason for a short time interval between the first collection of specimens at 9-13 gestational weeks and the second collection at 14-16 gestational weeks ? Of the 4038 participants, only 1076 completed the first sample collection. What was the impact on the overall study?

Response : We thank you for your positive comment regarding the IMPROvED cohort study. Specimens were collected at four time points throughout pregnancy to enhance the range of future research that can be conducted using IMPROvED biobank data. We have edited our Discussion to include this information as follows:

"IMPROvED contains both epidemiological data and biobank data that utilised numerous aliquots on multiple media enabling high quality research into the cause and prevention of adverse pregnancy outcomes. Specimens were collected at multiple time points throughout pregnancy to enhance the range of future research that can be conducted using the IMPROvED residual biobank."
The first study visit at 11+0 to 13+6 weeks' gestation was optional. Therefore, while participation at this first time-point was desirable, it was not mandatory, resulting in fewer completed samples than the second (mandatory) study visit (~15 weeks' gestation). This information is outlined in our Methods under 'Data collection and follow-up'.

2. *Second, the dietary pattern is also a contributing factor to preeclampsia, and the dietary habits vary among different countries. Whether dietary behaviours were considered for collection when designing this cohort study? If not, it is recommended to discuss in the limitation section.*

Response: We agree that poor dietary pattern is an important risk factor for preeclampsia. However, the IMPROvED cohort study did not collect data on dietary behaviours. We have edited our Limitations to include your suggestion, as follows:

"Data on dietary behaviours was not collected. Poor dietary patterns, before and during pregnancy, have been shown to increase the likelihood of preeclampsia and may be an important risk factor to consider in future research (1)."

We hope these amendments address the suggestions raised. Should any further amendments be necessary, we would be happy to address them accordingly.

Sincerely,

Dr Gillian Maher, Prof Louise Kenny, Dr Kate Navaratnam, Prof Zarko Alfirevic, Dr Darina Sheehan, Prof Philip Baker, Prof Christian Gluud, Dr Robin Tuytten, Prof Marius Kublickas, Dr Boel Niklasson, Prof Johannes Duvekot, Prof Caroline van den Berg, Prof Pensee Wu, Prof Karolina Kublickiene, Dr Fergus McCarthy, and Dr Ali Khashan

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1. Esquivel MK. Nutritional Status and Nutrients Related to Pre-Eclampsia Risk. American journal of lifestyle medicine. 2023;17(1):41-5.

Competing Interests: No competing interests were disclosed.