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Insulin sensitivity assessed using urine C peptide creatinine ratio (UCPCR) in pregnancy: cross-sectional analysis of an English multiethnic cohort

Ankica Markoska, Lilian C Mendoza, Rajalakshmi Valaiyapathi, Chloe Thorn, Anne Dornhorst

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Department of Investigative Medicine, Faculty of Medicine, Hammersmith Hospital, Imperial College London, London, UK

Correspondence to
Anne Dornhorst;
a.dornhorst@imperial.ac.uk

ABSTRACT

Aims To assess urinary C peptide creatinine ratio (UCPCR) used in a modified Matsuda equation to measure insulin sensitivity (IS) in pregnancy.

Research and design methods In this cross-sectional study, two IS measurements were calculated in 73 pregnant women at ~28 weeks of gestation by two separate methods using modified Matsuda equations. The first using the 0 and 120 min serum C peptide concentration during a 75 g oral glucose tolerance test (OGTT) and the second using the 0 and 120 min UCPCR values. The calculated IS measurements from the two methodologies were evaluated using Person's test and linear regression analysis. The relationship between IS_{OGTT} UCPCR and the fasting second void UCPCR and 120 min UCPCR was assessed using Pearson correlation and linear regression analysis after logarithmic transformation of the variables. Statistical analysis was performed using SPSS V.22.

Results The IS measured using serum C peptide ($IS_{OGTTc\text{-}pep}$) in the modified Matsuda equation correlated with the IS measurement using serum UCPCR ($IS_{OGTT\text{-}UCPCR}$) (r 0.704, p<0.0001). A strong correlation was found between the $IS_{OGTT\text{-}UCPCR}$ and the fasting UCPCR (r −0.916, p<0.0001), displaying a hyperbolic relationship.

Conclusion The UCPCR provides a practical methodology to assess IS and β-cell function in pregnancy.

Strengths and limitations of this study

- Urinary C peptide creatinine ratio (UCPCR) is a valid method to assess insulin secretion in and outside pregnancy. We are the first to report the use of UCPCR to assess insulin sensitivity in pregnancy using a modified Matsuda equation.
- A modified Matsuda equation using UCPCR provides a practical and non-invasive method to assess insulin sensitivity in pregnancy that could potentially be useful in epidemiological studies and clinical practice.
- We have observed a hyperbolic relationship between fasting UCPCR values and insulin sensitivity, suggesting that UCPCR could be used to estimate β-cell function.
- The study was conducted in pregnant women; therefore, the results cannot necessarily be extrapolated to a non-pregnant population.

INTRODUCTION

In pregnancy, maternal normoglycaemia is dependent on insulin secretion increasing sufficiently to compensate for the physiological fall in insulin sensitivity (IS). In clinical practice, measuring insulin secretion is relatively straightforward using serum insulin, serum C peptide or the urinary C peptide.[1–3] Urinary C peptide creatinine ratio (UCPCR), obtained using the fasting second-void urine sample, is strongly correlated with serum insulin, serum C peptide[4 5] and 24 hours urinary C peptide,[4] providing a practical and non-invasive method to assess insulin secretion. By contrast, measuring IS is much more complex. The euglycaemic hyperinsulinaemic clamp, although the gold standard, is impractical for clinical use. The Matsuda Index (IS_{OGTT}) provides a validated simpler alternative using serum glucose and insulin measurements during an oral glucose tolerance test (OGTT).[6] In pregnancy, the Matsuda Index exhibits a stronger correlation with the euglycaemic hyperinsulinaemic clamp, than other IS models (ie, HOMA-IR).[7] A modified Matsuda Index that substitutes serum C peptide for insulin has been validated during pregnancy.[8] Our previous work has shown that serum C peptide and UCPCR are strongly correlated during an OGTT in the latter half of pregnancy.[9] Using data collected during this study, we evaluate whether maternal UCPCR obtained during an OGTT can replace serum C peptide in the previous validated modified Matsuda Index of Radaelli *et al*.[8] We also evaluated the relationship between IS in the UCPCR-modified

Matsuda equation and insulin secretion estimated by the second-void fasting UCPCR.

Research design and methods

The present study is a further analysis of a published prospective cross-sectional study undertaken in the maternity unit at Queen Charlotte's and Chelsea Hospital, London, UK.[9] All women had given informed written consent. The original database was from 100 women prospectively recruited who agreed to provide an extra blood and urine sample during their routine diagnostic 28-week 75 g OGTT for gestational diabetes mellitus (GDM). Women were recruited over a 5-month period in 2016. All women were either 35 years old or above, expecting twins or had one or more risk factors for GDM according to the National Institute for Health and Care Excellence (NICE) guidelines.[10] All women included had normal renal function. From the original dataset of 100 women, 27 were excluded from the current analysis, 2 with gestational age above 31 weeks, 1 with a renal transplant and 21 with urinary C peptide above the assay detection limit after automated 1:10 dilution. A further three women were not included in the final analysis due to missing 120 min UCPCR data.

All women attended the 2-hour 75 g OGTT fasted and had passed their overnight first void urine. Fasting and 2-hour blood samples were taken for plasma glucose and serum C peptide. Urine samples were collected at the beginning (second void urine) and end of the OGTT.

The blood glucose was collected in fluoride oxalate tubes and processed in the routine hospital laboratory using the hexokinase/G-6-phosphate dehydrogenase spectrophotometric method, with an imprecision of ≤5% of the total coefficient of variation (CV), performed on Abbott Architect c System. The C peptide blood sample was collected on ice, before centrifugation and the plasma stored in 1 mL cryotubes at −80°C.

The urinary C peptide was collected and stored in boric acid tubes before being aliquoted into 1 mL cryotubes and stored at −80°C. Urinary creatinine was assayed using the kinetic alkaline picrate method with a total CV of ≤6% (Abbott Architect ci16200 System) to obtain the UCPCR.

Urinary and serum C peptide were measured on an Abbott Diagnostics Architect platform (Abbott Laboratories, Abbott Park, Illinois, USA), using a two-step chemiluminescent microparticle immunoassay, with measurement ranges between 3.33 and 10 000 pmol/L.

Box 1 Serum C peptide replaces serum insulin in the original Matsuda equation and 500 000 is used as the numerator

$IS_{OGTTC\text{-}pep}$=500 000/√{[FPGxFsC-pep] × [mean glucose × mean sC-pep during the OGTT]}

FPG, fasting plasma glucose (mg/dL); FsC-pep, fasting serum C peptide (pmol/L); IS, insulin sensitivity; OGTT, oral glucose tolerance test; sC-pep, serum C peptide (pmol/L).

Box 2 Urinary C peptide creatinine ratio (UCPCR) values at fasting and the mean UCPCR between 0 and 120 min replace the fasting and mean insulin values in the original Matsuda equation. 500 000 was also used here as the numerator

$IS_{OGTT\text{-}UCPCR}$=500 000/√{[FPG×FUCPCR] × [mean glucose × mean UCPCR during the OGTT]}

FPG, fasting plasma glucose; FUCPCR, fasting UCPCR (pmol/mmol); IS, insulin sensitivity; OGTT, oral glucose tolerance test.

The Architect C peptide assay was designed to have a precision of ≤10% total CV. Automated dilution of 1:10 was performed in urine samples exceeding the upper limit of assay detection.

Calculation of IS was made using two different modified Matsuda equations. The first equation used serum C peptide, as previously validated in pregnancy by Radaelli *et al*,[8] (box 1). In the second equation, IS was calculated replacing serum C peptide for UCPCR values (box 2).

Numeric data are presented as mean and SD, or median and IQR. Categorical data are shown as percentages. Data not normally distributed were

Table 1 Participants characteristics and oral glucose tolerance test (OGTT) results

Characteristics	%, mean (±SD) or median (IQR)	n
Maternal age (years)	34.1 (±4.7)	76
BMI (kg/m^2)	23.8 (5.8)	76
Ethnicity		76
Caucasian	51.3%	
South Asian	17.1%	
South East Asian	9.2%	
Middle Eastern	13.2%	
Black (African/Caribbean)	3.9%	
Other	5.3%	
Parity		76
Nulliparous	56.6%	
Multiparous	43.4%	
Singleton pregnancy (yes)	94.6%	76
Gestational age (weeks)	28.0 (0.28)	76
OGTT results		
Fasting glucose (mmol/L)	4.4 (0.5)	76
Fasting C peptide (pmol/L)	0.48 (0.29)	75
Fasting UCPCR (pmol/mmol)	2.93 (2.02)	75
2-Hour glucose (mmol/L)	5.4 (1.9)	76
2-Hour C peptide (pmol/L)	2.12 (1.14)	75
2-Hour UCPCR (pmol/mmol)	12.06 (11.4)	73
GDM (yes)	5.3%	76

BMI, body mass index; GDM, gestational diabetes mellitus; UCPCR, urinary C peptide creatinine ratio.

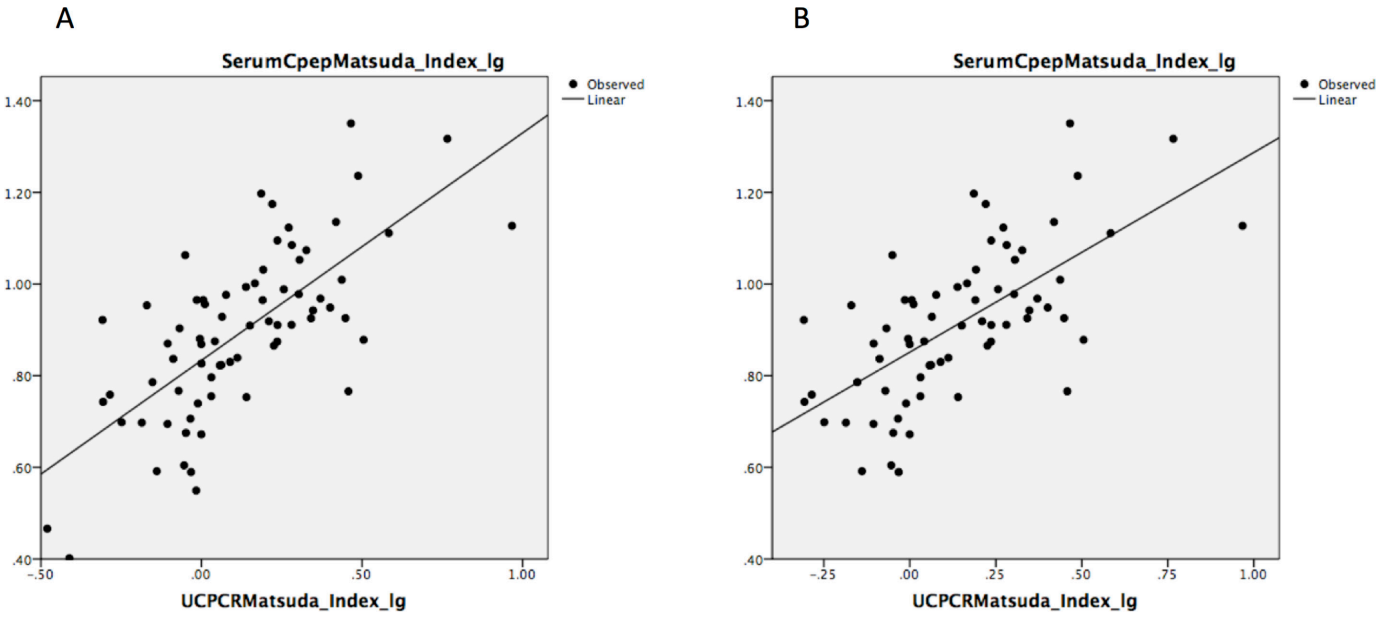

Figure 1 Linear regression models for $IS_{OGTTc\text{-}pep}$ and $IS_{OGTT\text{-}UCPCR}$: (A) in the full cohort: Y=0.833+0.496X, r=0.704, p<0.001 and (B) excluding women with gestational diabetes mellitus (GDM): Y=0.851+0.436X, r=0.654, p<0.001. IS, insulin sensitivity; OGTT, oral glucose tolerance test; UCPCR, urinary C peptide creatinine ratio.

log transformed for analysis. We evaluated the correlation between two modified Matsuda equations (boxes 1 and 2) using Pearson correlations and linear regression analysis. A sensitivity analysis was performed excluding the four women with GDM.

The relationship between insulin secretion and IS displays a hyperbolic function.[11 12] To verify if the OGTT-based measures of insulin secretion and IS in this study displayed a hyperbolic relationship, the following regression analysis was performed: log(secretion measure)=-constant + β × log(sensitivity measure). The criteria for a hyperbolic relationship were then applied: (1) β is approximately equal to −1 and (2) the 95% CI of β excludes 0.[12 13]

Statistical analysis was performed using SPSS V.22. A p value of <0.05 was considered statistically significant.

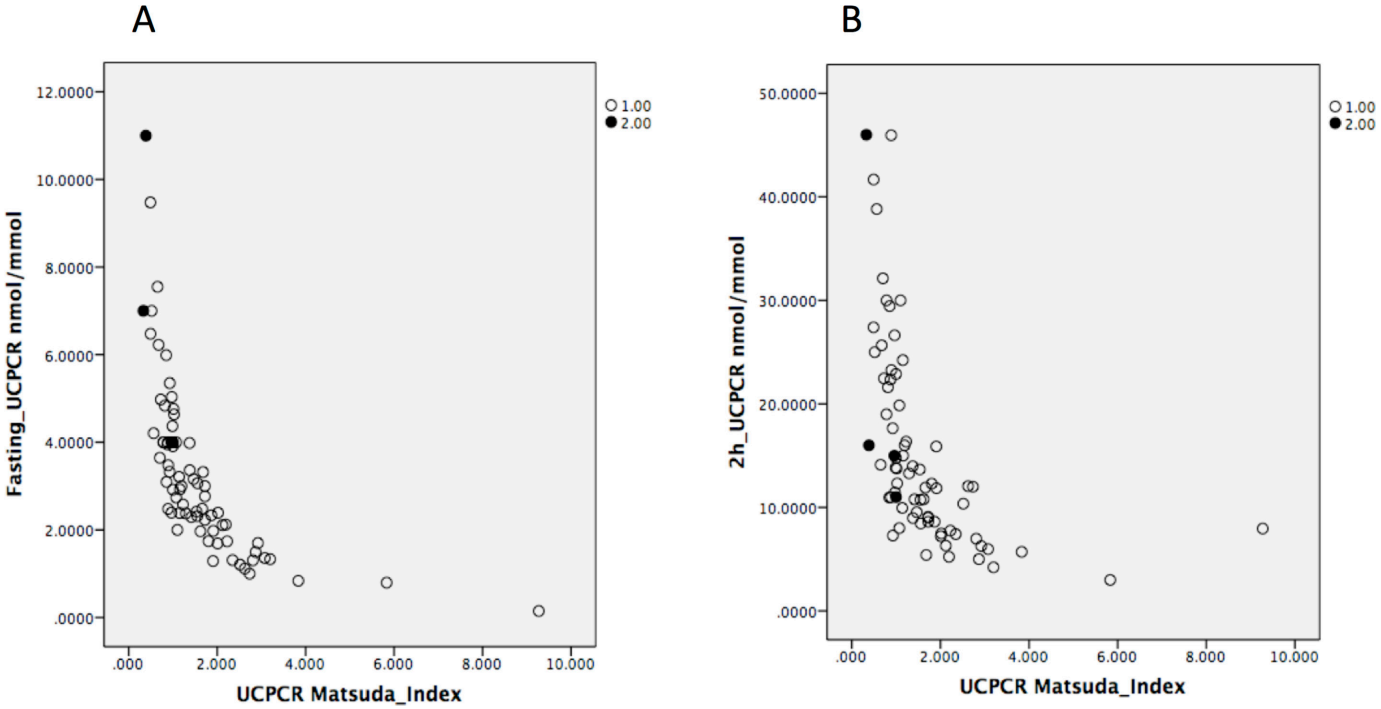

Figure 2 Correlation plot patterns between (A) $IS_{OGTT\text{-}UCPCR}$ and the fasting urinary C peptide creatinine ratio (UCPCR) and (B) $IS_{OGTT\text{-}UCPCR}$ and the 2-hour UCPCR. Women with gestational diabetes mellitus (GDM) are highlighted in black. IS, insulin sensitivity; OGTT, oral glucose tolerance test.

RESULTS

The 76 participant characteristics and their OGTT results are summarised in table 1.

The median estimates for $IS_{OGTTc\text{-}pep}$ and $IS_{OGTT\text{-}UCPCR}$ were 8.1 (2.5–22.4) and 1.2 (0.3–9.3), respectively.

The two IS indexes calculated ($IS_{OGTTc\text{-}pep}$ and $IS_{OGTT\text{-}UCPCR}$) were significantly correlated (r=0.704, p<0.0001) The linear regression model is summarised in figure 1A.

The association between $IS_{OGTTc\text{-}pep}$ and $IS_{OGTT\text{-}UCPCR}$ remained significant after excluding the four women with GDM in the sensitivity analysis (r=0.654, p<0.0001). The linear regression model is summarised in figure 1B.

The $IS_{OGTT\text{-}UCPCR}$ showed a strong correlation with both the fasting and the 120 min UCPCR (r=−0.916, p<0.0001) (r=−0.777, p<0.0001), respectively. A hyperbolic plot pattern was observed between $IS_{OGTT\text{-}UCPCR}$ and both, fasting and 120 min UCPCR (figure 2A, B). The four women who had GDM by NICE criteria fell on the left-hand side of this hyperbola in both graphs.

A hyperbolic relationship was displayed between $IS_{OGTT\text{-}UCPCR}$ and the fasting UCPCR (β −1.002, 95% CI −1.105 to −0.898, p<0.001) (figure 3A).

In the sensitivity analysis, excluding the four women with GDM, both of the hyperbolic criteria were consistent with the existence of a hyperbolic relationship between these two measures (β −1.019, 95% CI −1.132 to −0.907, p<0.001) (figure 3B).

However, the relationship between $IS_{OGTT\text{-}UCPCR}$ and the 120 min UCPCR did not fulfil the hyperbolic function criteria (β −0.767).

DISCUSSION

In this study, IS in pregnant women was calculated using two different modifications to the original Matsuda equations that replaced serum insulin in the original equation for either serum C peptide or UCPCR. The indexes calculated by both methods were well correlated. In pregnancy, the Matsuda equation using serum C peptide has been validated against the original method described by Matsuda using serum insulin.[8] Furthermore, in pregnancy, it has shown a better correlation with IS derived using the euglycaemic hyperinsulinaemic clamp than Homeostasis assessment model - insulin resistance (HOMA-IR).[7] This may be as HOMA-IR is highly dependent on basal hepatic insulin resistance[6] rather than total glucose disposal which will include maternal and fetal glucose uptake.

The use of the UCPCR provides a more convenient and practical method of assessing insulin secretion, as it is stable at room temperature for 3 days.[4] UCPCR has proven to be a robust tool to assess insulin secretion, having a good correlation with both circulating insulin concentrations and serum C peptide.[5] The fasting second-void urine UCPCR reflects an integrated measurement over time that has shown to be strongly correlated with 24 hours urinary C peptide measurements.[4] Our current results show that UCPCR provides an estimate of insulin secretion and it can, in a modified Matsuda equation, be used to assess IS.

A hyperbolic relationship was observed between $IS_{OGTT\text{-}UCPCR}$ and the fasting UCPCR, in our study. This mirrors the hyperbolic relationship that defines the Disposition Index (DI), the product of insulin secretion and IS,[11] which has been proposed as a measure of β-cell compensatory capacity, in both pregnant[14] and non-pregnant subjects.[12]

The utility of the DI as a measure of β-cell function was defined by the hyperbolic relationship between insulin secretion and IS derived from the intravenous glucose tolerance test.[15] More recently, this relationship has been

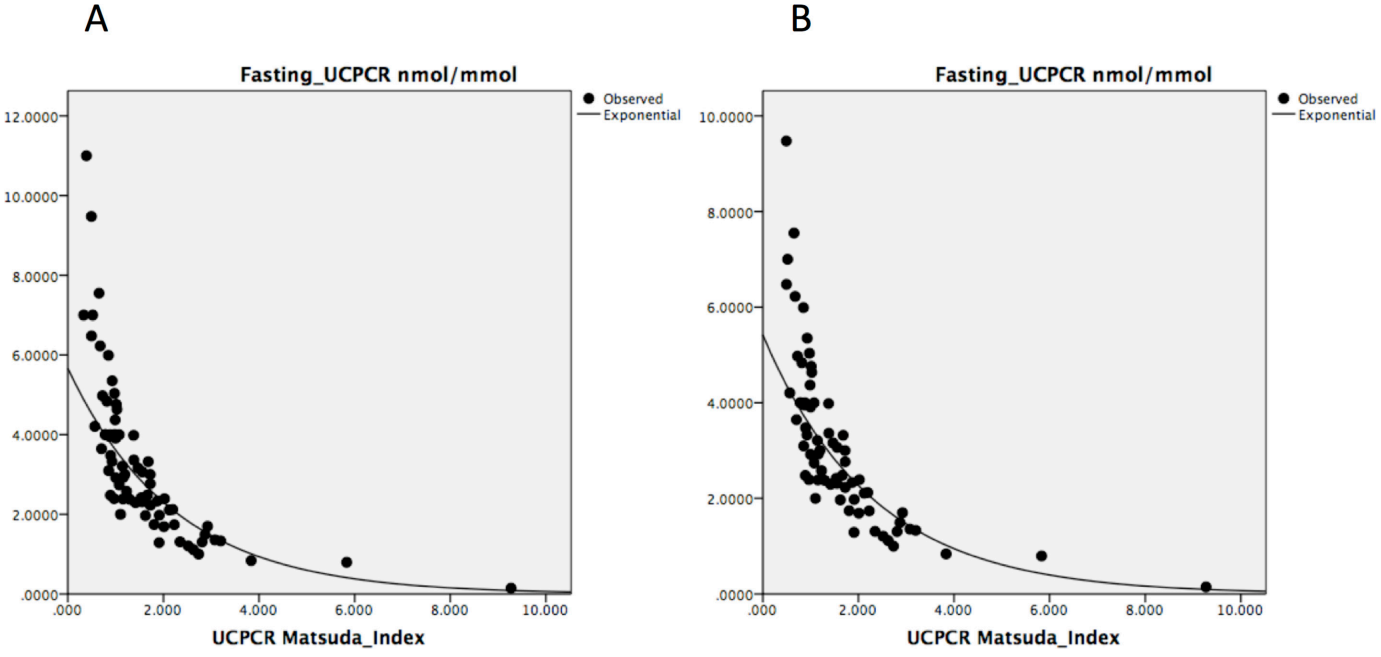

Figure 3 Hyperbolic relationship displayed between $IS_{OGTT\text{-}UCPCR}$ and the fasting urinary C peptide creatinine ratio (UCPCR): (A) in the full study cohort and (B) excluding the four women with GDM. IS, insulin sensitivity; OGTT, oral glucose tolerance test.

observed using parameters derived from the OGTT.[13] Our work suggests that UCPCR substituted into the Matsuda equation can also offer information regarding β-cell function during pregnancy.

It has been established that women entering pregnancy with decreased IS are more at risk of developing GDM.[16] UCPCR, as an easy and non-invasive clinical method to assess IS, could potentially help identify women most at risk of developing GDM in early pregnancy, allowing targeted lifestyle modification to lessen the risk of GDM. There is increasing evidence that lifestyle and therapeutic intervention after 18 weeks of pregnancy in at-risk women has little effect on preventing both GDM and fetal macrosomia.[17–19] By contrast, earlier intervention with diet and physical activity may have a beneficial role.[20]

A practical and accurate method to estimate IS in pregnancy could also be informative in better understanding the epigenetic impact of maternal obesity and glucose homeostasis on the fetus.[21 22]

A limitation of this study is that all women included had one or more risk factors for GDM. The study was conducted in pregnant women and therefore the results cannot necessarily be extrapolated to the non-pregnant population. Further studies on a larger scale are needed to corroborate these findings and confirm whether UCPCR could potentially be used to assess an individual's risk for developing GDM.

In summary, we have shown that the UCPCR-derived Matsuda Index in pregnancy is correlated with the serum C peptide-derived index, as validated by Radaelli *et al*. The relationship between IS estimated by the UCPCR-derived Matsuda equation and the fasting and post-OGTT UCPCR showed a hyperbolic relationship that suggests that this measurement could be useful to assess β-cell compensatory capacity in pregnancy.

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Contributors AM designed and conducted the study, analysed the data, interpreted the results and wrote the manuscript. LCM contributed to the data analysis, interpreting the results and writing of the manuscript. RV and CT contributed to the conduct of the study and data analysis. AD is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Imperial College Healthcare NHS Trust Tissue Bank and and the Research Ethics Committee Wales: 12/WA/0196.

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Data sharing statement Data supporting this study are stored by the corresponding author at Imperial College London and will be available on request.

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