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Risk of anaemia with metformin use in type 2 diabetes: A MASTERMIND study

Short title: Risk of anaemia with metformin use

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Abstract

Objective: To evaluate the association between metformin use and anaemia risk in type 2 diabetes, and the time-course for this, in randomised controlled trial (RCT) and real-world population data.

Research design and methods: Anaemia was defined as a haemoglobin measure less than 11g/dL. In RCTs (ADOPT (n=3,967), UKPDS (n=1,473)), logistic regression was used to model anaemia risk and non-linear mixed models for change in haematological parameters.In the observational GoDARTS population (n=3,485), discrete-time failure analysis was used to model the effect of cumulative metformin exposure on anaemia risk.

Results: In ADOPT, compared with sulfonylureas, the odds ratio(OR)(95%CI) for anaemia was 1.93(1.10,3.38) for metformin and 4.18(2.50,7.00) for thiazolidinediones. In UKPDS, compared with diet, the OR(95%CI) was 3.40(1.98,5.83) for metformin, 0.96(0.57,1.62) for sulfonylureas and 1.08(0.62,1.87) for insulin.

In ADOPT, haemoglobin and haematocrit dropped following metformin initiation by six months, with no further decrease after three years. In UKPDS, haemoglobin fell by three years in the metformin group compared to other treatments. At years six and nine, haemoglobin was reduced in all treatment groups, with no greater difference seen in the metformin group. In GoDARTS, each 1g/day of metformin use was associated with a 2% higher annual risk of anaemia.

Conclusions: Metformin use is associated with early risk of anaemia in individuals with type 2 diabetes, a finding consistent across two RCTs and replicated in one real-world study. The mechanism for this early fall in haemoglobin is uncertain, but given the time course is unlikely to be due to vitamin B12 deficiency alone.

INTRODUCTION

Anaemia is a common finding in individuals with type 2 diabetes (1). Metformin is the first-line therapy for treatment of type 2 diabetes in most individuals, and the most widely prescribed oral antidiabetic medication. A recent meta-analysis reviewed all available studies on associations between metformin use and vitamin B12 levels, anaemia, and neuropathy in individuals with type 2 diabetes (2). The meta-analysis confirmed individuals taking metformin had a significantly higher risk of vitamin B12 deficiency than those not taking metformin, and significantly lower serum B12 concentrations, which depended on dose and duration of treatment. Although this meta-analysis reported no association between metformin use and anaemia risk, it is important to note that there were only four eligible studies and most of these were cross-sectional or case-control which did not have anaemia as their primary endpoint (3-6). In contrast, the DPP Outcomes Study showed metformin use in individuals with impaired glucose tolerance was associated with an increased risk of anaemia at five years, independent of vitamin B12 status (7). There is therefore uncertainty about whether metformin causes anaemia, and whether or not this is mediated by B12 deficiency in metformin treated individuals with type 2 diabetes.

The aims of this study were firstly to use randomised controlled trial (RCT) data with repeated haematological measures to determine if there is an association between metformin use and anaemia risk in type 2 diabetes, and, if so, what is the time-frame for this? Secondly, to quantify risk in a real-world setting, by examining whether cumulative exposure to metformin is associated with an increase in incidence of anaemia using routinely collected clinical data.

RESEARCH DESIGN AND METHODS

Data sources

Three datasets were analysed: two from randomised controlled trials (A Diabetes Outcome Progression Trial [ADOPT], UK Prospective Diabetes Study **[**UKPDS] and one from routine clinical data Genetics of Diabetes Audit and Research in Tayside Scotland [GoDARTS]).

ADOPT was a trial in which 4,351 drug naïve individuals recently diagnosed with type 2 diabetes were assigned randomly to thiazolidinediones (TZDs), metformin or sulfonylurea monotherapy and followed up for five years (8). Haematological measures were collected at baseline, six months, one year and annually thereafter.

UKPDS was a trial in which 4,209 individuals with newly diagnosed type 2 diabetes were randomised to receive a conventional (diet) or intensive glycaemic management strategy (insulin, sulfonylurea or metformin) (9; 10). Haematological measures were collected at baseline, three, six and nine year follow up.

GoDARTS is a large population-based cohort of approximately 10,000 individuals with type 2 diabetes, with comprehensive electronic medical records containing detailed information on all encashed prescriptions (including daily dose and adherence) from 1994 onwards in Tayside and Fife, Scotland, as well as all routinely collected biochemistry and haematology measures (11).

Definition of anaemia

Haemoglobin (Hb) was the outcome variable. It was used as a continuous variable in ADOPT and UKPDS to investigate change over time, and recoded as a binary outcome for estimating the risk of anaemia in all three studies. The anaemia endpoint was defined as the first Hb measure less than 11g/dL in both males and females (the moderate anaemia definition used by the World Health Organization [WHO]) following diabetes diagnosis. Individuals with prevalent anaemia at baseline (diagnosis) (defined by WHO as Hb less than 12g/dL in females and 13g/dL in males) were excluded from this analysis.

Study Populations

ADOPT: All participants in the intention to treat subset were eligible for the study. Individuals with missing baseline covariates, or anaemia at baseline were excluded.

UKPDS: All participants with greater than 120% ideal body weight in the 15 centres recruited prior to 1988 were eligible for the study (9). Individuals with missing baseline covariates or anaemia at baseline were excluded.

GoDARTS: Individuals with type 2 diabetes diagnosed on or after 1st January 1996 were eligible for the study, thus ensuring sufficient prescribing information and Hb measurements. In addition, individuals were required to have a baseline Hb measure (defined as closest measure to type 2 diabetes diagnosis up to one year prior) and no anaemia at baseline. The study period for eligible individuals was defined as time from type 2 diagnosis (baseline) until first anaemia event, death, leaving area or end of follow-up (30th September 2015), whichever came first.

Statistical Analysis

ADOPT and UKPDS: The risk of moderate anaemia at any time point during the trials was modelled using logistic regression. The reference groups for treatment were sulfonylureas and diet for ADOPT and UKPDS, respectively. Covariates included in the model were sex, baseline age, Hb, eGFR and BMI.

Haematological changes over time were modelled using non-linear mixed models. Hb was modelled in ADOPT and UKPDS. Haematocrit (Hct) was modelled in ADOPT, and packed cell volume (PCV) was modelled in UKPDS as a proxy for Hct which was not available. Hb adjusted for Hct (ADOPT) or PCV (UKPDS) was also modelled to assess whether these were temporally related. In addition, mean corpuscular volume (MCV) was modelled in ADOPT. Data are presented as plots of predictions of the fixed effects for each treatment.

GoDARTS: Discrete time survival analysis was used to evaluate the effects of cumulative drug exposure. This model is set up as a logistic regression in which each individual contributes one observation for each 28-day time interval during the study period. A data matrix was generated with one row for each individual under observation in each time interval, and columns specifying event status (coded as binary), fixed and time dependent covariates at the start of each time interval.

Exposure to each diabetes drug class (metformin, sulfonylureas, insulin, TZDs, acarbose, glucagon-like peptide receptor agonist (GLP-1 RA), dipeptidyl peptidase-4 inhibitor (DPP4i), glinide and SGLT2i inhibitor (SGLT2i)) was calculated from the date and intended duration of each prescription, but gaps between prescriptions did not accumulate exposure, therefore adherence is accounted for in the model. Cumulative exposure of each drug class was calculated as the sum of all earlier intervals. If the drug was discontinued during the study the cumulative exposure was still carried forward to subsequent time intervals.

Age at diabetes diagnosis, sex, baseline Hb, calendar year of diabetes diagnosis and social deprivation (coded as 1-5 with 1 most deprived and 5 least deprived) were included as fixed covariates. Time from diabetes diagnosis was included as a time dependent covariate. In addition, we considered cumulative exposure to metformin in terms of total dose. BMI and eGFR were included as time dependent covariates in a sub group of individuals with these data available.

Pharmacoepidemiological studies are prone to allocation bias. To be satisfied that an estimate of a drug's potential causal effect cannot be due to time invariant between-person confounding we include two time-updated terms for each drug class: one for ever-exposure and one for cumulative exposure. We focus the inference of causality on the cumulative term (12). The terms for ever-exposure and cumulative exposure can be given a visual representation by plotting a regression line through the unadjusted rates of anaemia grouped by cumulative metformin exposure, representing the linear effect of cumulative exposure. The difference between the data point for the unexposed time (x=0) and the estimated regression line at this point gives the magnitude of the ever-exposed term. This is the sum of any immediate stepwise effect of metformin and any difference in prior anaemia risk in the never-users vs. ever-users of metformin.

GoDARTS analysis was conducted using SAS 9.4 software. ADOPT and UKPDS analyses were conducted using R software.

RESULTS

RCT datasets (ADOPT and UKPDS)

Study populations and anaemia rates

In ADOPT, from 4127 individuals in the intention-to-treat population, 153 were anaemic at baseline, and a further 7 had missing covariate data, leaving 3967 individuals in the study population (mean(sd) Hb 14.5(1.1) g/dL, age 56.6(10.0) with 58.9% male). There were 1,343 metformin, 1,289 sulfonylurea and 1,335 TZD users, with anaemia event rates of 38(2.8%), 19(1.5%) and 76(5.7%) respectively over the five-year follow up period.

In UKPDS, from 1704 individuals, 52 were anaemic at baseline, and a further 179 had missing covariate data, leaving 1473 individuals in the study population (mean(sd) Hb 15.1(1.2) g/dL, age 52.8 (8.1) with 47.1% male). There were 300 metformin, 461 sulfonylurea, 360 insulin and 352 diet treated individuals, with anaemia event rates of 19(6.3%), 5(1.1%), 9(2.5%) and 6(1.7%) respectively over the nine-year follow up period.

Logistic regression model for anaemia risk

The results of the logistic regression for risk of moderate anaemia are presented in supplementary tables 1 and 2 for ADOPT and UKPDS respectively. In ADOPT the adjusted odds ratio (OR), with sulfonylureas as the reference group, was 1.93(1.10,3.38) for metformin and 4.18(2.50,7.00) for TZDs. Other predictors of moderate anaemia risk were older age, lower baseline Hb and male sex.

In UKPDS the adjusted OR, with diet as the reference group, was 4.42(2.28,8.57) for metformin, 0.53(0.19,1.48) for sulfonylureas and 1.79(0.73,4.42) for insulin. In addition, lower baseline Hb was a predictor of moderate anaemia risk.

Non-linear mixed model for haemoglobin change over time

The plots of the prediction of the fixed effects from the non-linear mixed model for each treatment group over time are presented in **Figure 1**.

In ADOPT, there was an immediate drop from baseline (by first measurement at 6 months) in Hb (**Figure 1a**) in both the metformin and TZD arms. The effect was much larger with TZD treatment but the metformin treatment arm followed a similar pattern. Hct fell in a similar pattern to that seen for Hb after both metformin and TZD treatment (**Figure 1b**), and this reduction in Hct completely mediated the fall in Hb (**Figure 1c**). There was no further Hb decrease in ADOPT between 3 and 5 years; at 5 years mean Hb was 0.42 (0.20,0.65) g/dL lower in the metformin treated arm than the sulfonylurea treated arm. There was a significant downward linear trend in MCV over the 5 years in the metformin treatment arm ($p<0.0001$) but no significant trend in the TZD or sulfonylurea arms (**Supplementary Figure 1**).

In UKPDS, there was also a post-baseline reduction in Hb when first measured (at 3 years) in those randomised to metformin compared with all other treatments (**Figure 1d**), with the Hb 0.49(0.41,0.57) g/dL lower that those diet treated. Hb fell in all treatment groups at years 6 and 9 but with no greater further fall seen in the metformin vs. diet treated group (0.49(1.64, 2.62) vs. (0.50(1.71,2.72) g/dL fall from 3 to 9 years). Similar to ADOPT, the PCV (proxy for Hct) also fell with metformin treatment (**Figure 1e**), and adjustment for the fall in PCV largely ameliorated the fall in Hb (**Figure 1f**).

Routine Clinical Dataset (GoDARTS)

Derivation of study population

From a total of 6440 individuals, 3765(58%) had a baseline Hb measure. Of these, 280 were excluded as they were anaemic at diagnosis, leaving 3485 individuals for analysis. A comparison of characteristics of individuals included and excluded in the study is presented in Supplementary Table 3. Individuals excluded due to missing baseline Hb measure were younger, with a higher proportion of males. However, individuals excluded due to anaemia at baseline were older, with a higher proportion of females. The final study population was older than the overall type 2 diabetes population (mean(sd) 62.7(10.6) vs. 61.8(11.0) years at diagnosis respectively, p=0.0005) with no difference in proportion of males and females (55.5% males in final study population), and mean(sd) Hb 14.7(1.2) g/dL.

Comparison of exposed and unexposed individuals

Of the 3485 individuals in the study, 2487 had accumulated some exposure to metformin by the end of follow-up. Table 1 shows the comparison of characteristics at diabetes diagnosis between exposed and unexposed individuals. Ever-users of metformin were younger, more socially deprived, with a higher proportion of males, higher Hb, BMI and eGFR.

Study period and outcome

A total of 1458 (41.8%) individuals had a moderate anaemia event during the follow up period: 745 in current users, 194 in ex-users and 519 in never-users. The median (IQR) follow up time was 8.3(5.0,11.5) years, number of Hb measures per individual in the model was 11(6,20) and frequency of measures was 7.6(4.5,13.5) months.

Effects of cumulative metformin exposure

Figure 2 shows the unadjusted rates of anaemia by cumulative exposure to metformin standardised (within 10-year age bands) to the age distribution (over all person-years) of the whole study population. With increasing cumulative exposure there is a higher risk of moderate anaemia in metformin users, which is linear after an initial high rate in the first year. As ever-users were younger with higher Hb at diagnosis (Table 1) it is unlikely that this group of individuals were at higher prior risk of anaemia. It is more likely that this initial greater risk of anaemia with metformin can be attributed to an immediate effect of metformin on Hb, particularly in light of the ADOPT data where we see a significant change at 6 months (**Figure 1a**).

Discrete-time failure model

The results for the discrete time failure analysis are presented in Table 2. In a simple model (model 1) with no diabetes drugs included, older age, longer duration of diabetes, lower baseline Hb, and higher social deprivation were associated with higher anaemia risk. There was no difference by sex or calendar year of diagnosis, so these covariates were not included in subsequent models. In model 2a, cumulative exposure and ever-exposure to metformin were added. The OR per year of cumulative exposure to metformin was 1.05(1.02,1.08). In model 2b cumulative metformin exposure is expressed as total dose (1.02(1.01,1.04) per one year of 1 gram per day). The results of adjusting for all diabetes drug classes are presented in model 3, and the association of cumulative metformin with moderate anaemia risk remains. In addition, there was an association between cumulative exposure to SGLT2i inhibitors and lower risk of moderate anaemia (OR 95%CI 0.46[0.36,0.59]).

For a subgroup of patients where longitudinal measures of BMI (n=3335) and eGFR (n=2920) were available, these were added to the model as time dependent covariates. BMI was not

significantly associated with moderate anaemia risk (data not shown). However lower eGFR was associated with higher moderate anaemia risk (model 4).

DISCUSSION

In this study we show for the first time that metformin use is associated with risk of moderate anaemia in individuals with type 2 diabetes, and that this finding is consistent across two RCTs and replicated in one real-world study of routinely collected data. Furthermore, in the large, observational, population-based study with a maximum follow-up period of almost 20 years, we show that each 1g/day of metformin use was associated with a 2% higher risk of moderate anaemia per year.

Moderate anaemia risk with metformin treatment in ADOPT and UKPDS

In the ADOPT study we observe the well-described early reduction in Hb seen with initiation of TZD treatment (an effect seen pre-marketing and included in the summary of product characteristics https://www.medicines.org.uk/emc/medicine/4236). We observe a similar pattern in the metformin treated group in ADOPT with an early fall in Hb, with no subsequent change after the first 2 years. These early changes in Hb (translated into moderate anaemia events) are matched by findings, albeit observational as opposed to trials, in the real world GoDARTS data. Similarly, in UKPDS the main difference in Hb between the metformin and other treatment arms had occurred by the first measurement at 3 years. The finding in ADOPT, and to a large extent in UKPDS, that the fall in Hct (PCV) mirrors the fall in Hb is consistent with the anaemia being caused by either a reduction in red cell mass, or an increase in plasma volume, or both. The fall in Hct (and Hb) seen with the TZDs is usually attributed to fluid retention and haemodilution, although other mechanisms have been proposed, such as a reduction in erythropoiesis due to either a direct effect on the bone marrow or secondary to lowering insulin levels (13). For metformin, it is not possible to infer a mechanism for the early reduction in Hb we observed in the data we had access to. In UKPDS, we show no treatment-specific effect of metformin on plasma sodium, albumin, urea, white blood cell

count, or aspartate amino transferase (AST) (data not shown) that might collectively point to haemodilution, bone marrow suppression or haemolysis. However, it seems unlikely that the mechanism for these early changes in Hb is secondary to B12 deficiency, as individuals should have enough B12 stored to last for between two to five years (3). Furthermore, the MCV during the 5 year ADOPT study did not increase with metformin treatment (**Supplementary Figure 1**), and in the GoDARTS study, of those who developed anaemia in the metformin exposed group compared with the non-metformin exposed group, microcytic anaemia was more frequent (12.1% vs. 7.3%) and macrocytic less frequent (7.6% vs. 12.3%) (data not shown), suggesting that the anaemia is not caused by B12 deficiency.'

Predictors of moderate anaemia risk in GoDARTS

In the GoDARTS discrete-time failure model we confirm the known predictors of anaemia risk in type 2 diabetes, namely older age, longer duration of diabetes, lower baseline Hb and lower eGFR (measured time dependently) adding external validity to our model.

The association between cumulative SGLT2i exposure and an apparent 'protective' effect on anaemia risk is in line with RCT findings from The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) study (14), which showed that Hct increases soon after initiation of SGLT2i therapy, and remains elevated for duration of treatment. Elevation of Hct has generally been interpreted as indicating haemoconcentration due to the diuretic effect of SGLT2i.

It is perhaps surprising given the ADOPT results that cumulative exposure of TZD was not associated with anaemia risk in GoDARTS. This is most likely an artifact of the model, given metformin is the first line drug for most individuals, and the study outcome is time to incident anaemia, therefore susceptible individuals are more likely to experience an event during their metformin exposure and drop out of the study before starting TZDs.

Overall anaemia rates

The overall moderate anaemia rates for the GoDARTS, ADOPT and UKPDS studies were 41.8, 3.4 and 2.2% respectively. The obvious reasons for the large difference between the real-world data and the trials are the GoDARTS population are older at diagnosis and followed up for longer with a mean(sd) age at diagnosis 62.7(10.6) vs. 52.8 (8.1) and 56.6 (10.0) in UKPDS and ADOPT respectively, and a median (IQR) study duration of 8.3(5.0,11.5) compared with a maximum 5 years follow up in ADOPT and 9 years in UKPDS. In addition, Hb was measured more frequently in the GoDARTS population due to the observational nature of the study, where all routinely collected measures were included, resulting in a median(IQR) number of Hb measures per individual of 11(6, 20) compared with a maximum of 4 and 7 in UKPDS and ADOPT respectively. Thus increasing the chances of a moderate anaemia event being detected. In addition, those patients in ADOPT or UKPDS who develop anaemia do not automatically drop out of the analysis and could be treated, thus potentially explaining the difference between the persisting risk of anaemia with metformin in the observational GoDARTS study compared to in the ADOPT RCT.However, it is important to note that in the GoDARTS study we only included 54% of the population, as a baseline Hb was required (characteristics of included and excluded individuals are provided in Supplementary Table 3) and these individuals would be expected to have been at a greater risk of anaemia (by virtue of it being requested by health care professional) and so the overall rate may be an overestimate for the general population.

Limitations

The main limitation of the reported studies is the lack of B12 measurement and lack of other data to help point to a mechanism mediating the early reduction in Hb caused by metformin treatment. Careful studies, assessing water balance and red cell production and turnover are warranted to better understand how metformin is causing a reduction in Hb.

A post hoc analysis of RCT data may be considered a limitation. However, in the absence of a specifically designed prospective study there are very few RCTs of metformin, ADOPT and UKPDS are perhaps the best two trials of sufficient size and quality to address this question.

Conclusions

In this study, including data from two RCTs, albeit post hoc, we have shown that metformin consistently causes an early reduction in Hb and increases rates of moderate anaemia. The absolute Hb reductions are not large (0.5g/dL at 5 years with ADOPT, 0.5g/dL at 3 years with UKPDS) although this does translate to a large increase in moderate anaemia rates, with an overall effect in the real-world population study of a 2% increase risk of anaemia per year per 1g/day of metformin; greater in the first year. As the mechanisms for metformin-related moderate anaemia are unknown, the effects are modest and the benefits of metformin are proven, we would not in any way advocate avoidance or discontinuation of metformin, even in patients with anaemia, but a reduction in Hb in the first few years after initiation of metformin might be anticipated.

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Duality of Interest

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No other potential conflicts of interest relevant to this article were reported.

Author Contributions

LAD, JMD, RLC, NS, ATH, RRH and ERP designed the study. LAD, JMD and RLC performed the analyses. LAD and ERP wrote the manuscript. JMD, RLC, NS, ATH and RRH reviewed/edited the manuscript.

Prior Presentation

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Table 1: Comparison of characteristics at diabetes diagnosis between those never-users and eventual users of metformin

	Metformin user during study		
	Never	Ever	*P*
n	998	2487	
Age (years)	68.3(10.0)	60.5(10.1)	<.0001
% males	50%	57.7%	<.0001
Year of diagnosis	2005(4.5)	2003.4(3.8)	<.0001
Social deprivation:			
1 (most deprived)	26.1%	27.7%	
2	20.5%	22.2%	
3	15.1%	16.6%	
4	19.4%	16.9%	
5	18.9%	16.7%	0.0301
Hb (g/dL):			
All	14.3(1.2)	14.8(1.2)	<.0001
Males	15.0(1.1)	15.3(1.1)	<.0001
Females	13.7(1.0)	14.1(1.0)	<.0001
BMI(kg/m^2)	30.6(5.7)	32.5(6.1)	<.0001
eGFR (ml/min per 1.73^2)	75.2(20.4)	87.5(18.3)	<.0001

Data are presented as means(sd) or %; Comparisons are t-test for continuous and chi-square test for categorical variables

Table 2: Discrete-time failure models for moderate anaemia in GoDARTS

	OR	95% CI	*P*
Model 1:			
Age at diagnosis (per 1 year)	1.05	1.04,1.05	**<.0001**
Time from diagnosis (per 1 year)	1.11	1.09,1.12	**<.0001**
Calendar year of diagnosis (per 1 year)	0.99	0.98,1.01	0.8405
Hb at diagnosis (per 1g/dL)	0.71	0.67,0.75	**<.0001**
Females (vs. Males)	1.05	0.94,1.18	0.3841
Social deprivation (per 1 category (most deprived lowest))	0.96	0.93,0.99	**0.0285**
Model 2a:			
Age at diagnosis (per 1 year)	1.05	1.04,1.06	**<.0001**
Time from diagnosis (per 1 year)	1.08	1.06,1.10	**<.0001**
Hb at diagnosis (per 1g/dL)	0.70	0.66,0.73	**<.0001**
Social deprivation (per 1 category (most deprived lowest))	0.96	0.93,0.99	**0.0396**
Ever metformin	1.12	0.98,1.28	0.1045
Cumulative metformin (per 1 year)	1.05	1.02,1.08	**0.0002**
Model 2b:			
Age at diagnosis (per 1 year)	1.05	1.04,1.06	**<.0001**
Time from diagnosis (per 1 year)	1.08	1.06,1.10	**<.0001**
Hb at diagnosis (per 1g/dL)	0.69	0.66,0.73	**<.0001**
Social deprivation (per 1 category (most deprived lowest))	0.96	0.93,0.99	**0.0366**
Ever metformin	1.16	1.02,1.32	**0.0224**
Cumulative metformin dose (per 1 year of 1g daily)	1.02	1.01,1.04	**0.0001**
Model 3:			
Age at diagnosis (per 1 year)	1.05	1.04,1.06	**<.0001**
Time from diagnosis (per 1 year)	1.07	1.05,1.09	**<.0001**
Hb at diagnosis (per 1g/dL)	0.69	0.65,0.73	**<.0001**
Social deprivation (per 1 category (most deprived lowest))	0.96	0.93,0.99	**0.0440**
Ever metformin	1.09	0.95,1.25	0.2217
Ever sulfonylurea	1.04	0.90,1.21	0.5668
Ever TZD	1.05	0.83,1.32	0.6768
Ever insulin	1.43	1.12,1.83	**0.0043**
Ever GLP-1 RA	0.51	0.25,1.03	0.0588
Ever DPP4i	0.83	0.58,1.18	0.2953
Ever SGLT2i	0.55	0.08,3.96	0.5562
Ever glinide	0.89	0.40,2.01	0.7825
Ever acarbose	0.82	0.37,1.82	0.6225
Cumulative metformin (per 1 year)	1.06	1.03,1.08	**<.0001**
Cumulative sulfonylurea (per 1 year)	1.01	0.98,1.04	0.5404
Cumulative TZD (per 1 year)	0.99	0.92,1.07	0.9082
Cumulative insulin (per 1 year)	0.98	0.93,1.04	0.5254
Cumulative GLP-1 RA (per 1 year)	1.10	0.85,1.44	0.4689
Cumulative DPP4i (per 1 year)	0.95	0.77,1.17	0.6307
Cumulative SGLT2i (per 1 year)	0.46	0.36,0.59	**<.0001**
Cumulative glinide (per 1 year)	1.02	0.70,1.48	0.9307
Cumulative acarbose (per 1 year)	0.91	0.58,1.42	0.6710
Model 4:			
Age at diagnosis (per 1 year)	1.03	1.02,1.04	**<.0001**
Time from diagnosis (per 1 year)	1.05	1.03,1.08	**<.0001**

Hb at diagnosis (per 1g/dL)	0.70	0.66,0.74	**<.0001**
Social deprivation (per 1 category (most deprived lowest))	0.97	0.93,1.01	0.1906
eGFR (per 1ml/min per 1.73^2)	0.98	0.98,0.99	**<.0001**
Ever metformin	1.16	0.99,1.37	0.0760
Ever sulfonylurea	0.99	0.84,1.18	0.9451
Ever TZD	1.04	0.80,1.36	0.7593
Ever insulin	1.31	0.99,1.72	0.0525
Ever GLP-1 RA	0.71	0.32,1.57	0.3992
Ever DPP4i	1.00	0.67,1.50	0.9947
Ever SGLT2i	0.96	0.13,6.96	0.9644
Ever glinide	0.55	0.20,1.50	0.2428
Ever acarbose	0.91	0.41,2.03	0.8132
Cumulative metformin (per 1 year)	1.06	1.02,1.09	**0.0006**
Cumulative sulfonylurea (per 1 year)	1.00	0.97,1.04	0.8184
Cumulative TZD (per 1 year)	0.98	0.90,1.07	0.6533
Cumulative insulin (per 1 year)	0.99	0.93,1.05	0.6753
Cumulative GLP-1 RA (per 1 year)	1.06	0.79,1.43	0.6876
Cumulative DPP4i (per 1 year)	0.88	0.69,1.11	0.2754
Cumulative SGLT2i (per 1 year)	0.40	0.26,0.62	**<.0001**
Cumulative glinide (per 1 year)	1.20	0.83,1.72	0.3352
Cumulative acarbose (per 1 year)	0.86	0.54,1.36	0.5102

Figure legends

Figure 1: Plots of haematological changes over time using non-linear mixed models. Data are presented as plots of predictions of the fixed effects for each treatment at each study visit. A: ADOPT trial: Hb. B: ADOPT trial: Hct. C: ADOPT trial: Hb adjusted for Hct. D: UKPDS trial: Hb. E: UKPDS trial: PCV. F: UKPDS trial: Hb adjusted for PCV

Figure 2: Plot of unadjusted rates of anaemia by cumulative exposure to metformin standardised (within 10-year age bands) to the age distribution (over all person-years) of the whole study population. The terms for ever-exposure and cumulative exposure were given a visual representation by plotting a regression line through the unadjusted rates of anaemia grouped by cumulative metformin exposure, representing the linear effect of cumulative exposure.