Methods

Study settings and referral criteria

The study was conducted in the Maternal-Fetal Medicine Unit of the Sant'Anna University Hospital (150 beds for obstetric patients) in Turin, and in the Nephrology Department of the Brotzu Hospital (a general hospital with 30 beds for Nephrology and Transplantation patients, and 25 beds in Obstetrics) in Cagliari, Italy.

These two settings have the broadest and longest experience with CKD in pregnancy in Italy, and patients with kidney diseases in pregnancy in both of these settings have been followed-up by a team of Nephrologists and Obstetricians. In Turin, the out-patient Unit dedicated to kidney diseases in pregnancy was set up in the year 2000 in the Maternal-fetal hospital, and was managed by the same Obstetrics and Nephrology team until June 2013, when the Nephrology team was sub-divided into three main referral Units, one in each main city hospital, all of which refer to the same Obstetrics Unit (34). Patients are referred to the Obstetrics Unit by the Nephrology Departments in the region, by family physicians and by the local counseling obstetrics centers.

In Cagliari, a joint Nephrology-Obstetrics outpatient service dedicated to patients with kidney diseases in pregnancy has been active since 1989 in the Nephrology department. The same Nephrologist has been in charge of the Unit since it was set up, and it is closely linked to the Nephrology outpatient service dedicated to glomerular diseases. The Unit mainly cooperates with the Obstetrics Department of the same hospital, that is the main referral source; patients are also referred by other regional Nephrology departments, by various Obstetrics Units and by counseling centers and family physicians.

Both Units collected all the relevant data prospectively (including serum creatinine, and Clearances or proteinuria based on 24 hour urine collection). The databases were updated and merged on December 31st, 2013, and data concerning all the cases that had been referred since January 1st, 2000, were selected.

Patient and control population

The patients.

The present study included CKD patients with singleton pregnancies and of gestational age >23 completed

weeks (this was considered the minimum limit for viability, consistent throughout the period); consequently, miscarriages and pregnancy terminations were excluded (<23 gestational weeks and/or weight <500 g). Further reasons for exclusion were: on-going pregnancy; multiple pregnancies; preeclampsia without evidence of underlying CKD (these patients were more frequently referred to the Cagliari Unit, while only patients requiring differential diagnosis with CKD were referred to the Turin Unit). Altogether, only 13 patients were lost to follow-up, all of whom were in Turin. This was most likely due to the different social structure of the two settings. Turin and its surroundings have about 1 million inhabitants, 8 Nephrology Units-Departments in the city and 4 in the surrounding areas, and 6 Obstetrics Departments; Cagliari and surroundings have about 350,000 inhabitants and 1 Nephrology Department with hospitalization ward and 3 Obstetrics Departments.

Overall, 508 pregnancies out of 731 referred pregnancies (including 4 intrauterine deaths and 504 singleton deliveries) were taken into consideration for the present analysis (Figure 1).

The controls.

The controls were selected from three sources, which will be referred to as Turin (made up of Turin 1 and Turin 2) and Cagliari. The three cohorts include low-risk cases, defined as pregnancies occurring in the absence of hypertension, obesity, diabetes, CKD, cardiovascular diseases or any other disease or condition potentially affecting pregnancy outcomes. The only exception was previous thyroid disease, or well controlled hypothyroidism on chronic thyroxin therapy, on account of the high frequency of thyroid disorders in the Italian population, especially in Sardinia, and of the known influence of hypothyroidism on conception rates, but not on pregnancy outcomes (36, 38-39).

Turin 1 is a historical control group of "low-risk pregnancies" followed-up between 1999 and 2007. It consists of a cohort of singleton, low-risk pregnancies that were cared for in the same Maternal-Fetal Unit. Turin 2 is a more recent control group of low risk pregnancies selected from the list of mothers enrolled in the European Intergrowth study, on the basis of their delivery/pregnancy termination at the Turin University hospital. All the patients that were referred between 2011 and 2013, for whom data concerning delivery (or pregnancy termination) were available were selected. Since no difference in baseline data or in outcomes was found in the

two control groups, they were merged into a single one.

The Cagliari cohort consists of a control group retrieved from the files of the Brotzu Hospital (the main Regional hospital). They were selected from among the low-risk patients who were followed-up by the Obstetrics Outpatient Unit between 2009 and 2013.

Three sample months were randomly selected per year (opaque sealed envelopes) and all pregnancies occurring in that period were recorded.

We initially considered 879 low-risk singleton pregnancies, and after excluding miscarriages and patients lost to follow-up, 839 cases were selected for comparison, including 3 intrauterine deaths and 836 singleton deliveries (Figure 2).

Definitions

GFR measurement

CKD was classified according to K-DOQI guidelines (37); eGFR calculation was based on pre-conception data, when available within 3 months prior to conception or on data at first check-up in pregnancy (; the calculation was performed by) Cockroft and Gault, MDRD and CKD-EPI formulas were used for the calculations, with the latter being chosen in the present study on account of its more widespread use (36, 41-42).

Preconception data, within 3 months from referral, were available in 38% of the cases in Torino and in 57% of the cases in Cagliari. After referral, creatinine clearance and proteinuria were assessed on 24 hour urine collection.

Diagnostic categories

The diagnoses of CKD were classified as: glomerulonephritis, diabetic nephropathy, Systemic Lupus Erythematosous (SLE) and collagen diseases or vasculitides, interstitial nephropathy, single kidney, kidney transplantation, renal malformations, previous acute pyelonephritis (with scars), polycystic kidney disease, isolated urinary anomalies (such as microhematuria or isolated non nephrotic proteinuria), other, or unknown, and were recorded as reported in the Nephrology charts. Furthermore, the diagnoses were stratified into two

main categories: systemic diseases (including diabetic nephropathy, SLE, collagen diseases or vasculitides, kidney transplantation) and all other chronic kidney diseases. All diagnoses were reviewed by the two senior nephrologists (GBP and GC) (36).

Obstetric definitions

Hypertension was defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90, or antihypertensive therapy; patients on anti-hypertensive therapy prior to conception were considered "hypertensive" even when antihypertensive therapy was discontinued during pregnancy.

Pre-eclampsia (PE) was strictly defined as hypertension accompanied by proteinuria ≥300 mg/24 hours after 20 weeks of gestational age in a previously normotensive, non-proteinuric woman in the absence of other signs or symptoms indicating a different nephrological diagnosis; the presence of Doppler flow alterations was considered further proof of PE diagnosis. Since the definition of "superimposed PE" (PE superimposed on hypertension or proteinuria already present at baseline) is not unequivocal, we did not use it in this study.

A newborn was defined as small for gestational age (SGA) when birth weight was below the 10^{th} percentile or according to Italian birth weight references (Parazzini scale and INeS charts) (28-29). The Parazzini scale, which was the Italian referral scale until 2012, was chosen for multivariate analysis since its use as a referral scale covers most of our study period. Preterm delivery was defined as before 37 completed gestational weeks; early-preterm delivery was defined as before 34 gestational weeks (35).

Prenatal and intrapartum care

In both settings, prenatal and intrapartum care of low-risk pregnancies followed the current guidelines (43-44) and the frequency of nephrological and obstetric visits for CKD patients was tailored to the patient and ranged from 1 visit every 4-6 weeks in non hypertensive, non proteinuric non-systemic CKD stage 1 patients to 1-2 times weekly in patients with severe proteinuria or hypertension or CKD stages 4-5, alone or combined.

At each clinical consultation, blood pressure was measured at least once and weight was recorded; fetal growth was checked by serial measurements of symphysis fundal height. Ultrasound biometry and Doppler study of

uterine and umbilical arteries were individualized.

The clinical policies were overall similar in both settings; therapeutic blood pressure goal was ≤130/80. Drugs of choice were Nifedipine or α-methyldopa. Beta blockers or Doxazosine were employed in case of insufficient response or severe side effects with the above mentioned drugs. Hospitalization was required in the presence of poorly controlled hypertension, worsening of renal function, new onset proteinuria or rapid worsening, severe upper urinary tract infection, and for any other problem regarding the mother and/or the fetus.

In singleton pregnancies, the aim was to delay delivery at least until term (37 weeks); indications for early delivery included severe worsening of maternal and/or fetal conditions up to the 32nd week of gestational age or less severe worsening after 32 weeks; worsening of the maternal conditions including PE, HELLP syndrome, poorly controlled hypertension, rapidly increasing nephrotic proteinuria or creatinine, alone or in combination. Fetal worsening included abnormal fetal heart rate tracings at any gestational age, absent end diastolic flow velocities at Doppler study of the umbilical arteries at or after 32 weeks of gestational age, no fetal growth for over two weeks at later gestational ages. Cesarean section was performed for fetal indications, before or during labor, or in cases of unfavorable conditions for, or lack of response to, induction. The indications for admission to the (Neonatal Intensive Care Unit - NICU were: birth weight <1800 g, gestational age <34 weeks, need for intubation, other severe or potentially severe disease or condition requiring 24 hour monitoring and/or intensive care (such as sepsis or severe hypoglycemia**).**

Statistical analysis

CKD data were collected prospectively in both Centers, and were periodically entered into electronic databases. The two archives were updated and merged for the 2000-2013 study period.

The following data were gathered and/or calculated: for all patients and controls: Center; date of referral and delivery; age, parity, race, educational level, BMI, gestational age at delivery, type of delivery, clinical complications, fetal weight, Centile, Apgar index, sex of the baby, admission to ICU, outcome; for CKD patients, serum creatinine, GFR, e-GFR, CKD stage, kidney disease, previous follow-up.

Since no maternal or neonatal deaths occurred and only 4 intrauterine deaths were observed in the study cohort,

we limited our analysis to the following outcomes: preterm delivery (<37 and <34 weeks), small for gestational age baby (SGA)) , admission to the Neonatal Intensive Care Unit (NICU), cesarean section.

Since the different outcomes are interrelated, we analyzed two combined outcomes: a "general" one, including pre-term delivery, need for NICU and SGA, and a "severe" one, combining early preterm-delivery with the need for NICU and SGA. The combined outcome did not include cesarean sections to avoid leveling off the data (in particular for the early-preterm, almost inevitably born by cesarean section).

A descriptive analysis was performed as appropriate (mean and standard deviation for parametric data; median and range for non-parametric data). Paired T-test, Chi-square test, Fisher's test, Kruskal-Wallis test, Mann-Whitney U test, ANOVA and T- test with Bonferroni were used for comparisons between cases and controls and among groups. A descriptive analysis was performed as appropriate. Multivariate logistic regression analysis was used to check for simultaneous effects of covariates. Adjusted odds ratio and 95% confidence intervals were derived from the estimated regression coefficients.

The logistic regression analyses included: in CKD stage 1: systemic diseases; baseline hypertension; baseline proteinuria >=1 g/day (this latter cut-point was chosen as it is less likely to identify a pregnancy-related disorder when assessed at baseline) (45).

The Odds Ratios derived from the analysis of the combined outcomes in different subsets of stage 1 patients were plotted as a Forest plot, according to the analysis performed separately for the two settings (Turin and Cagliari) and then combined according to a random effect model.

The Hosmer–Lemeshow test was employed as a measure of goodness of fit. Models for which expected and observed event rates in subgroups are similar are considered "well calibrated": the lack of statistical significance confirms the good fit of the model.

Statistical analyses were performed with SPSS vers18.0 for Windows (SPSS Chicago Ill, USA). Significance was set at <0.05.