Cytoreductive therapy prevents renal dysfunction associated with primary myelofibrosis

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Abstract

In patients with myeloproliferative neoplasms (MPNs), a variety of symptoms are observed that are at least partly, if not completely, induced by altered levels of cytokines owing to oncogenic expansion of hematopoietic cells. In the present study, we performed a single-center study to investigate the relationship between renal dysfunction, which is thought to be promoted by altered levels of cytokines, and MPNs. We retrospectively analyzed 121 patients with MPNs before they started to receive treatment against MPNs, and found that the median estimated glomerular filtration rate (eGFR) at initial diagnosis in patients with primary myelofibrosis (PMF) was lower than in general population and in patients with polycythemia vera (PV) or essential thrombocythemia (ET) (PV vs PMF: P = 0.039, ET vs PMF: P = 0.007). This implied that renal function was preferentially impaired in PMF. Furthermore, PMF patients harboring *JAK2* V617F mutation exhibited more rapid progression of renal dysfunction judged by the reduction rate of eGFR during the disease course compared to those with *CALR* exon 9 mutation (P = 0.014). Progression of renal dysfunction in PMF patients that were treated with a cytoreductive reagent was significantly attenuated compared to that in patients that were not treated with the reagent (P = 0.016). We also found that more rapid progression of renal dysfunction in patients with MPNs was associated with uric acid level at initial diagnosis (P = 0.019). Collectively, our study demonstrated that cytoreductive therapy may prevent the progression of renal dysfunction in patients with PMF.

Introduction

Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), exhibit expansion of one or more of the myeloid lineage cells caused by an acquired driver mutation, such as *JAK2* V617F, *CALR* exon 9, or *MPL* W515K/L [1]. It has been shown that hematopoietic cells harboring these mutations exhibit not only increased growth potential but also change the production of various cytokines in the body [2, 3], which is thought to cause a variety of symptoms associated with MPNs. Such systemic abnormality may increase the burden on renal organ and promote renal dysfunction with aging. In agreement with this, it has been suggested that MPNs are potentially associated with renal dysfunction [4-6]. However, due to a limited number of studies, it is unclear whether a specific type of MPNs is associated with renal dysfunction; whether driver mutation affects the renal dysfunction; and whether the treatment against MPNs affects the renal dysfunction in the disease course. In the present study, we performed a single center study with a large MPNs cohort to address these questions.

Material and methods

Patients

Patients visited our hospital before receiving any therapy and diagnosed as MPNs based on the WHO 2008 criteria were studied. Driver mutation was determined as described previously[7-10]. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Juntendo University School of Medicine. When patients were grouped by treatment history, those who had received the treatment regardless of the term were considered as treated.

Data analysis

Renal function was evaluated by the estimated glomerular filtration rate (eGFR) calculated based on the Japanese Society of Nephrology formula: eGFR (mL/min/1.73m^2) = 194 × Cr$^{-1.094}$ × age$^{-0.287}$ × (0.739 if female , 1 if male)[11]. The Modification of Diet in Renal Disease formula was not used as it has been shown that this formula was not applicable to Japanese population[12]. To study changes of the

eGFR during the disease course, eGFR values from the following data points were analyzed: the first diagnosis; the first visit between 3 to 5 months after the first diagnosis; the first visit 6 months after the previous data point up to 3 years since the first diagnosis; and the first visit 12 months after the previous data point. If patients' data were not available for the time points defined by the above criteria, they were left blank for subsequent analyses.

Statistics

Categorical variables and continuous variables (Table 1) were analyzed using the chi-squared test and Kruskal-Wallis test, respectively. To evaluate renal function at the time of initial diagnosis, the Wilcoxon's rank-sum test, and Benjamini and Hochberg's multiple comparison procedures were used. False discovery rate (FDR)-adjusted P-values are presented in Figure 1A. To assess the renal function in patients with different MPN subtypes grouped by driver mutation, an ordinary least squares regression model adjusting for age was used. FDR-adjusted P-values are presented in Figure 1 C–E for multiple comparisons. To investigate the change of the renal function in patients during the course of the disease, a mixed-effect repeated measures model depending on the group defined by MPN subtype and mutation status, smoking, duration from initial diagnosis for eGFR, and interaction between the group and the duration with a consideration of the following covariance structures: unstructured, compound symmetric, and first-order autoregressive. Covariance structures that provided the best fit according to the Akaike's information criterion are depicted in Figures 2 A–C, and 3 A, B. To investigate the association between laboratory data at initial diagnosis and the change of renal function in the disease course (Table 2), a mixed-effect repeated measures model that depended on each subject in Table 2, duration from initial diagnosis for eGFR, group defined by MPN subtypes and mutation status, and interaction between the group and the duration was used in a similar manner to the above analysis. All statistical analyses were performed using R (version 3.4.1, R Foundation for Statistical Computing, Vienna, Austria). All tests were two-sided, and values of $P <$ 0.05 and FDR-adjusted $P < 0.05$ were considered statistically significant.

Results

Reduced renal function in patients with PMF

To investigate renal function in patients with MPNs, clinical records of 121 patients who visited our hospital before receiving any treatment against MPNs were collected and analyzed. Prior medication, including low-dose aspirin for diseases other than MPN, was not considered due to limited availability of the records. Based on WHO 2008 criteria, 23, 76, and 22 patients were diagnosed with PV, ET, or PMF with median eGFR values at initial diagnosis of 77.80 mL/min/1.73m^2, 80.55 mL/min/1.73m^2, and 70.40 mL/min/1.73m^2, respectively (Table 1). The median eGFR in patients with PMF was lower than in general population (75 mL/min/1.73m^2)[13] or in patients with PV or ET (PV vs. PMF: P = 0.039; ET vs. PMF: P = 0.007) (Figure 1A). Even after the adjustment for age, eGFR in patients with PMF was significantly lower than in patients with PV or ET (PV vs. PMF: P = 0.026, ET vs. PMF: P = 0.026). Consistent with these data, there was a trend that the frequency of chronic kidney disease defined by the eGFR below 60 mL/min/1.73 m^2 was nominally higher in patients with PMF (27.3%) than in patients with PV (13.0%) or ET (14.5%) (Figure 1B).

Driver mutation did not correlate with renal dysfunction at initial diagnosis

Because driver mutation status correlates with the clinical features in MPNs[10, 14-16], we investigated whether renal function impairment was affected by the driver mutation status. In PV, no significant differences were observed when median eGFR values at initial diagnosis were compared between the carriers of *JAK2* V617F, *JAK2* exon 12, and triple-negative individuals (*JAK2* V617F vs. *JAK2* exon 12: P = 0.39, *JAK2* V617F vs triple-negative: P = 0.762; *JAK2* exon 12 vs. triple-negative: P = 0.39) (Figure 1C). In ET and PMF, no significant differences were observed when median eGFR values at initial diagnosis were compared in carriers of *JAK2* V617F or *CALR* exon 9 mutations, and triple-negative individuals (*JAK2* V617F vs. *CALR* exon 9: P = 0.614, *JAK2* V617F vs. triple-negative: P = 0.18, *CALR* exon 9 vs. triple-negative: P = 0.18) (Figure 1D), or *JAK2* V617F and *CALR* exon 9 (P = 0.199) (Figure 1E).

Progression of renal dysfunction in patients with PMF harboring *JAK2* V617F

To investigate the change of renal function throughout the disease course,

eGFR values in patients with each type of MPNs were determined. As shown in Figure 2A, no significant difference in the time-dependent change of renal function was observed between MPN subgroups (P = 0.618). The same analysis was further performed when patients with ET and PMF were grouped by the driver mutation status. Note that PV was excluded from this analysis due to insufficient number of patients with *JAK2* exon 12 mutation (n = 3) and triple-negative status (n = 3) (Table 1). Although no significant differences were observed between driver mutation subgroups of ET (Figure 2B, P = 0.344), patients with PMF harboring *JAK2* V617F exhibited a significantly higher rate of renal dysfunction progression compared to those harboring *CALR* exon 9 mutation (Figure 2C, P = 0.014). There was no analysis of triple-negative PMF due to the insufficient number of patients in this group (n = 1) (Table 1). These results implied that *JAK2* V617F promotes renal dysfunction in patients with PMF but not in patients with ET.

Suppression of renal dysfunction during the disease course in patients with PMF by cytoreductive therapy

To investigate the effect of the anti-MPN therapy on the progression of renal dysfunction, the changes in renal function throughout the course of the disease were compared among patients grouped by the MPN subtype and the treatment history. As shown in Figure 3A, no significant effect on the change in eGFR during the disease course was observed between patients with different disease subtypes regardless of a history of low-dose aspirin treatment. Note that those patients with PMF that received low-dose aspirin treatment exhibited a trend of accelerated decrease of eGFR compared to those who did not receive the treatment; however the effect did not reach statistical significance (Figure 3A, P = 0.259). PMF patients with a history of hydroxyurea treatment showed significant improvements in eGFR compared to those without such treatment (Figure 3B, P = 0.016). This relationship, however, was not evident in patients with PV or ET (Figure 3B). These results indicated that cytoreductive therapy suppressed renal dysfunction during the disease course in patients with PMF.

Uric acid level at initial diagnosis was associated with the progression of renal dysfunction

Finally, to identify parameters that distinguish individuals who have a higher risk to develop renal dysfunction among patients with MPNs, we examined the relationship between laboratory data at initial diagnosis and the change of renal function afterwards. As shown in Table 2, the higher uric acid level was significantly associated with the progression of renal dysfunction in the disease course (P = 0.019).

Discussion

Here, we have investigated renal dysfunction in 121 patients with MPNs in a single-center retrospective study and found that: 1) renal function of PMF patients was significantly decreased compared that in patients with PV or ET (Figure 1A); 2) driver mutation did not affect renal function at initial diagnosis (Figure 1C–E); 3) presence of *JAK2* V617F was associated with progressive renal dysfunction in patients with PMF (Figure 2C), which may explain why the survival of PMF patients harboring this mutation is overall shorter than in patients with *CALR* exon 9 mutation[14]; 4) cytoreductive therapy prevented the progression of renal dysfunction in patients with PMF (Figure 3B), and 5) the level of uric acid, which induces endothelial dysfunction[17, 18], at initial diagnosis predicted the progression of renal dysfunction in the disease course (Table 2).

In contrast to previous studies in Denmark [4] and Korea [5], where eGFR values at diagnosis in patients with PMF were similar to or better than in patients with ET or PV, respectively, we found that eGFR values at initial diagnosis in PMF patients were significantly lower than those in patients with ET or PV. Furthermore, we found that hydroxyurea treatment suppressed the progression of renal dysfunction in patients with PMF, which was not evident in the previous studies [4, 5]. These discrepancies may be due to the following reasons: 1) in the previous studies, patients may have received treatment against MPNs at diagnosis; 2) in the previous studies, non-MPN patients may have been included due to the use of WHO 2001 criteria; and 3) variable treatment regimens may have been used in different regions and times.

Pronounced renal dysfunction in PMF patients may be a consequence of renal fibrosis [19] recognized as glomerulosclerosis and tubulointerstitial fibrosis, which can be induced by PDGF and TGF-β[20, 21] that are aberrantly increased in PMF

[22, 23]. Alternatively, renal extramedullary hematopoiesis, the association of which with PMF has been previously described[24], may induce renal dysfunction by disorganizing the tissue. Regardless of the cause of renal dysfunction in patients with PMF, this pathology was prevented by hydroxyurea treatment that suppresses tumor cell activity.

Our study was based on the creatinine level that can be influenced by muscle mass, vigorous exercise, and high intake of meat. Therefore, more direct assessment of renal function, e.g., by measuring urinary protein and urine occult blood at initial diagnosis, is required for better understanding of the renal dysfunction in patients with MPNs. In addition, effects of more advanced therapies, e.g., with a JAK2 inhibitor, on renal dysfunction should be examined.

In conclusion, we found that patients with PMF exhibited renal dysfunction, which was likely to progress in *JAK2* V617F-positive patients in the disease course. Because renal dysfunction leads to renal anemia, it is important to prevent the progression of the former in patients with PMF. Cytoreductive therapy apparently prevented the progression of renal dysfunction in patients with PMF. Based on these findings, we propose that PMF patients harboring *JAK2* V617F mutation need more frequent checkups for renal function and recommend considering the use of cytoreductive therapy to prevent progression of renal dysfunction.

Conflict of interest

The authors declare no competing interests

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Figure legends

Figure 1: Renal function in patients with myeloproliferative neoplasms at initial diagnosis.

A. A box-whisker plot of estimated glomerular filtration rate (eGFR) in patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) at initial diagnosis. **B.** Pie charts illustrate frequencies of chronic kidney disease (CKD) at initial diagnosis. Definition of CKD was the presence of eGFR below 60mL/min/1.73m^2. **C–E**. Dot plots of eGFR at initial diagnosis in patients with PV (**C**), ET (**D**), and PMF (**E**) grouped by the driver mutation status.

Figure 2: Change of renal function in the disease course in patients with myeloproliferative neoplasms (MPNs).

A. Regression lines of eGFR values during the disease course in patients with MPNs. The rate of change was not significantly different in patients with polycythemia vera (PV, blue), essential thrombocythemia (ET, red), and primary myelofibrosis (PMF, green). NS: not significant. **B**. Regression lines of eGFR values during the disease course in patients with ET grouped by the driver mutation status, such as *JAK2* V617F (JAK2, green), *CALR* exon 9 (CALR, blue), and triple-negative (TN, red). NS: not significant. **C**. Regression lines of eGFR values during the disease course in patients with PMF grouped by the driver mutation status, such as *JAK2* V617F (JAK2, green) and *CALR* exon 9 (CALR, blue). Triple-negative patients were omitted from this analysis due to the small number of patients (*n* = 1).

Figure 3: Cytoreductive therapy prevented the progression of renal dysfunction in patients with PMF.

A. Regression lines of eGFR values during the disease course in patients with different MPN subtypes that either received (+) or did not receive (−) low-dose aspirin treatment. NS: not significant. **B.** Regression lines of eGFR values during the disease course in patients with different MPN subtypes that either received (+) or did not receive (−) hydroxyurea treatment.

Table 1. Patients' characteristics.

Diagnosis	PV (N=23)	ET (N=76)	PMF (N=22)	Combined (N=121)	P value
Age	62.00 (49.50, 71.50)	59.00 (44.00, 71.25)	65.00 (56.00, 71.75)	60.00 (48.00, 72.00)	0.243
Gender (female)	13 (56.5%)	44 (57.9%)	7 (31.8%)	64 (52.9%)	0.09
eGFR	77.80 (70.30, 93.35)	80.55 (66.58, 96.38)	70.40 (54.08, 78.48)	77.80 (65.30, 93.20)	0.008
HT	11 (47.8%)	24 (31.6%)	7 (31.8%)	42 (34.7%)	0.34
DM	1 (4.3%)	10 (13.2%)	3 (13.6%)	14 (11.6%)	0.484
IHD	4 (17.4%)	5 (6.6%)	1 (4.5%)	10 (8.3%)	0.201
DVT	1 (4.3%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	0.117
Other thrombosis	0 (0.0%)	1 (1.3%)	0 (0.0%)	1 (0.8%)	0.742
Stroke	2 (8.7%)	6 (7.9%)	3 (13.6%)	11 (9.1%)	0.71
Other-kidney disease	0 (0.0%)	1 (1.3%)	2 (9.1%)	3 (2.5%)	0.083
Smoking/Prior smoking	6 (30.0%)	25 (38.5%)	5 (41.7%)	36 (37.1%)	0.744
Phlebotomy	18(78.3%)	3 (3.9%)	0 (0.0%)	21(17.4%)	ND
Anagrelide	0 (0.0%)	16 (21.1%)	0 (0.0%)	16 (13.2%)	ND
Ruxolitinib	1 (4.3%)	0 (0.0%)	4 (18.2%)	5 (4.1%)	ND
Hydroxyurea	10 (43.5%)	25 (32.9%)	4 (18.2%)	39 (32.2%)	ND
Low-dose aspirin	16 (69.6%)	35 (46.1%)	2 (9.1%)	53 (43.8%)	ND
JAK2 V617F	17(73.9%)	36 (47.4%)	13(59.1%)	66(54.5%)	ND
JAK2 exon12	3(13%)	0(0.0%)	0(0.0%)	3(2.5%)	ND
CALR exon9	0 (0.0%)	19 (25.0%)	8 (36.4%)	27(22.3%)	ND
MPL W515K/L	0 (0.0%)	1 (1.3%)	0 (0.0%)	1 (0.8%)	ND

Values at first diagnosis except for the treatment and mutation status are presented as frequencies with percentages for categorical variables and medians with interquartile ranges for continuous variables.

PV: polycythemia vera, ET: essential thrombocythemia, PMF: primary myelofibrosis, HT: hyper tension, DM: diabetes mellitus, IHD: ischemic heart disease, DVT: deep-vein thrombosis, ND: not-determined.

Table 2. Association between laboratory data at initial diagnosis and the progression of renal dysfunction in the disease course.

	Regression coefficient	Std Error	*P* value
WBC	0.0	0.00	**0.481**
Neutrophil	0.0	0.00	**0.298**
Monocyte	0.0	0.01	**0.231**
RBC	0.0	0.06	**0.735**
Hb	-3.0	5.05	**0.547**
PLT	0.1	0.07	**0.148**
UA	-3.7	1.57	**0.019**
LDH	0.0	0.01	**0.513**

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, PLT: platelet, UA: uric acid, LDH: lactate dehydrogenase
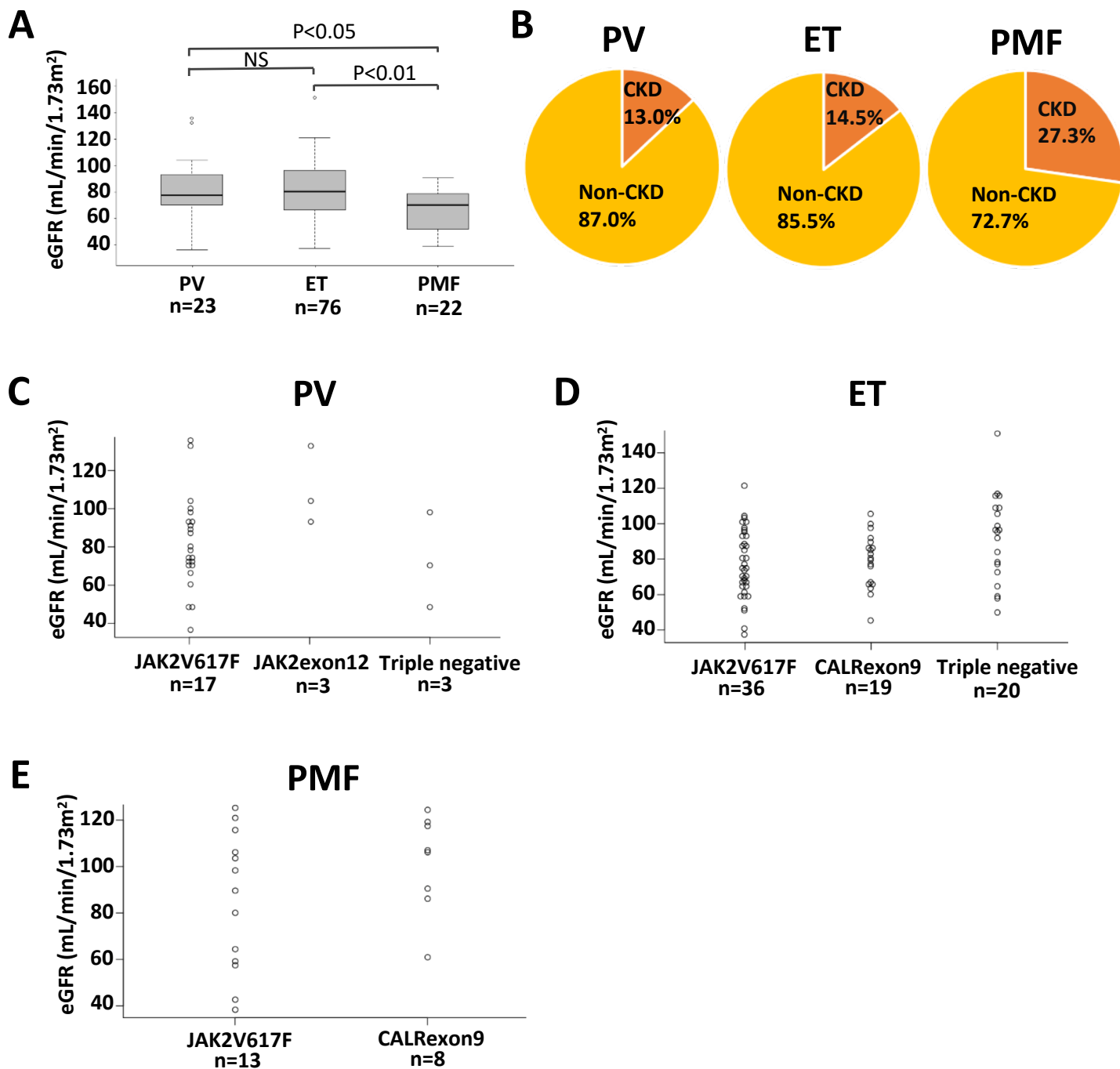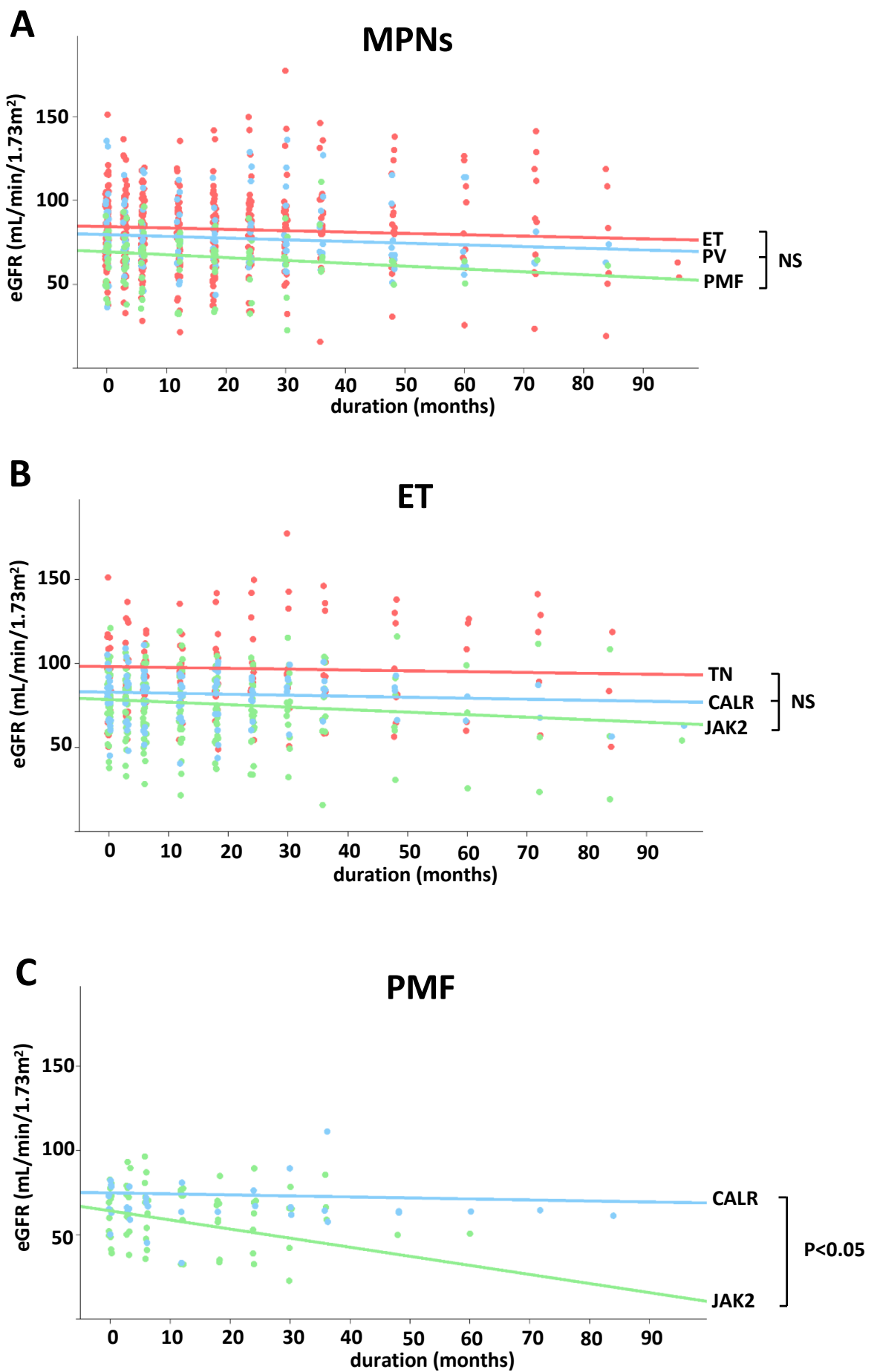
Figure 1

Figure 2

Figure 3

A — Low-dose aspirin

eGFR (mL/min/1.73m²) vs duration (months)

ET -
PV +
ET +
PMF -
PV -
NS

PMF +

B — Hydroxyurea

eGFR (mL/min/1.73m²) vs duration (months)

ET -
PV -
PV +
ET +
PMF +
P<0.05

PMF -