Evidence for prevention of renal dysfunction associated with primary myelofibrosis by cytoreductive therapy

Myeloproliferative neoplasms (MPN), such as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are characterized by the expansion of myeloid lineage cells caused by an acquired driver mutation, such as *JAK2*V617F, *CALR* exon 9, or *MPL* W515K/L.[1] Hematopoietic cells harboring these mutations exhibit increased growth potential and altered production of various cytokines,[2] which causes a variety of symptoms associated with MPN. Such systemic abnormality may increase the burden on kidneys and promote renal dysfunction with aging. Indeed, MPN are potentially associated with renal dysfunction.[3-5] However, it has not been clarified as to whether a specific type of MPN is associated with renal dysfunction, whether the driver mutation affects renal dysfunction, and whether treatment of MPN affects renal dysfunction. In the present study, we performed a single center study with a large cohort of patients with MPN to address these questions.

Clinical records of 121 patients who visited our hospital before receiving any treatment against MPN were collected and analyzed (*Online Supplementary Methods*). Prior medication, including low-dose aspirin administration for diseases other than MPN, was not considered owing to limited availability of the records. Based on World Health Organization (WHO) 2008 criteria, 23, 76, and 22 patients were diagnosed with PV, ET, or PMF, with median estimated glomerular filtration rate (eGFR) values of 77.80 mL/min/1.73 m^2, 80.55 mL/min/1.73m^2, and 70.40 mL/min/1.73m^2, respectively, at initial diagnosis (*Online Supplementary Table S1*). Unlike in previous studies, where the eGFR values at diagnosis in patients with PMF were similar to or better than those in patients with ET or PV,[3,4] the median eGFR in patients with PMF in our cohort was lower than in the general population (75 mL/min/1.73m^2)[6] or in patients with PV or ET (PV *vs.* PMF: P=0.039; ET *vs.* PMF: P=0.007) (Figure 1A). Even after adjusting 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). The discrepancies may be explained by the fact that in previous studies, the patients may have received treatment against MPN at diagnosis and/or non-MPN patients may have been included because of the use of WHO 2001 criteria. Nevertheless, in our cohort, the frequency of chronic kidney disease (CKD) defined by eGFR value below 60 mL/min/1.73 m^2 was nominally higher in patients with

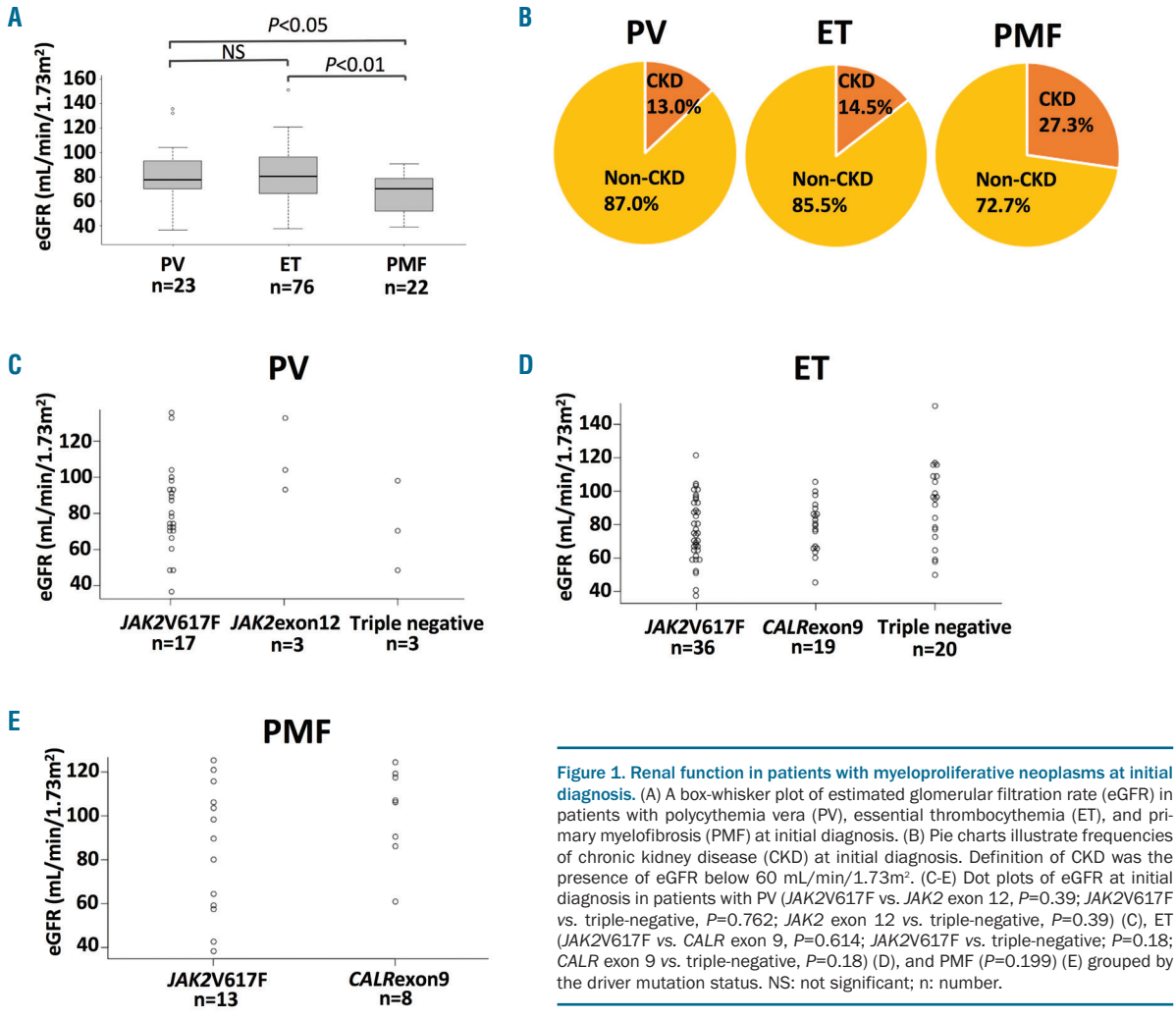

Figure 1. Renal function in patients with myeloproliferative neoplasms at initial diagnosis. (A) A box-whisker plot of estimated glomerular filtration rate (eGFR) in patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) at initial diagnosis. (B) Pie charts illustrate frequencies of chronic kidney disease (CKD) at initial diagnosis. Definition of CKD was the presence of eGFR below 60 mL/min/1.73m^2. (C-E) Dot plots of eGFR at initial diagnosis in patients with PV (*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) (C), ET (*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) (D), and PMF (P=0.199) (E) grouped by the driver mutation status. NS: not significant; n: number.

PMF (27.3%) than in patients with PV (13.0%) or ET (14.5%) (Figure 1B).

Because driver mutation status correlates with MPN clinical features,[7] we determined driver mutations as described previously,[8] and found that it did not significantly affect median eGFR values at initial diagnosis in PV, ET, or PMF cases (Figure 1C-E).

Furthermore, MPN subtype did not statistically alter eGFR values throughout the disease course (Figure 2A). The same analysis was also performed when ET and PMF patients 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) (*Online Supplementary Table S1*). Although eGFR was not significantly different in ET patients with different driver mutations (Figure 2B), patients with PMF that harbored *JAK2*V617F exhibited a significantly higher rate of renal dysfunction progression than those harboring *CALR* exon 9 mutation (P=0.014) (Figure 2C). Effect of the triple-negative status on PMF was not analyzed as the group comprised only one patient (*Online Supplementary Table S1*). These results suggested that *JAK2*V617F promotes renal dysfunction in patients with PMF but not in patients with ET. Although eGFR values at initial diagnosis were on the whole similar in patients with different mutation status for most MPN subtypes, renal function was significantly reduced in *JAK2*V617F-positive PMF patients. This may be owing to more progressive pathology in such patients than in patients with *CALR* exon 9 mutation.[7]

To investigate the effect of 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 treatment history. No significant effect on eGFR change during the disease course was observed among patients with different disease subtypes, regardless of the history of low-dose aspirin treatment (Figure 3A). Patients with PMF with a history of hydroxyurea (HU) treatment showed significant eGFR improvements compared to those without such treatment (P=0.016) (Figure 3B). This relationship, however, was not evident in patients with PV or ET (Figure 3B). HU treatment likely reduced chronic inflammatory load in PMF patients, which improved eGFR. However, no significant reduction in C-reactive protein level was observed in PMF patients who received HU treatment compared to those who did not (*data not shown*). Nevertheless, these results strongly suggested

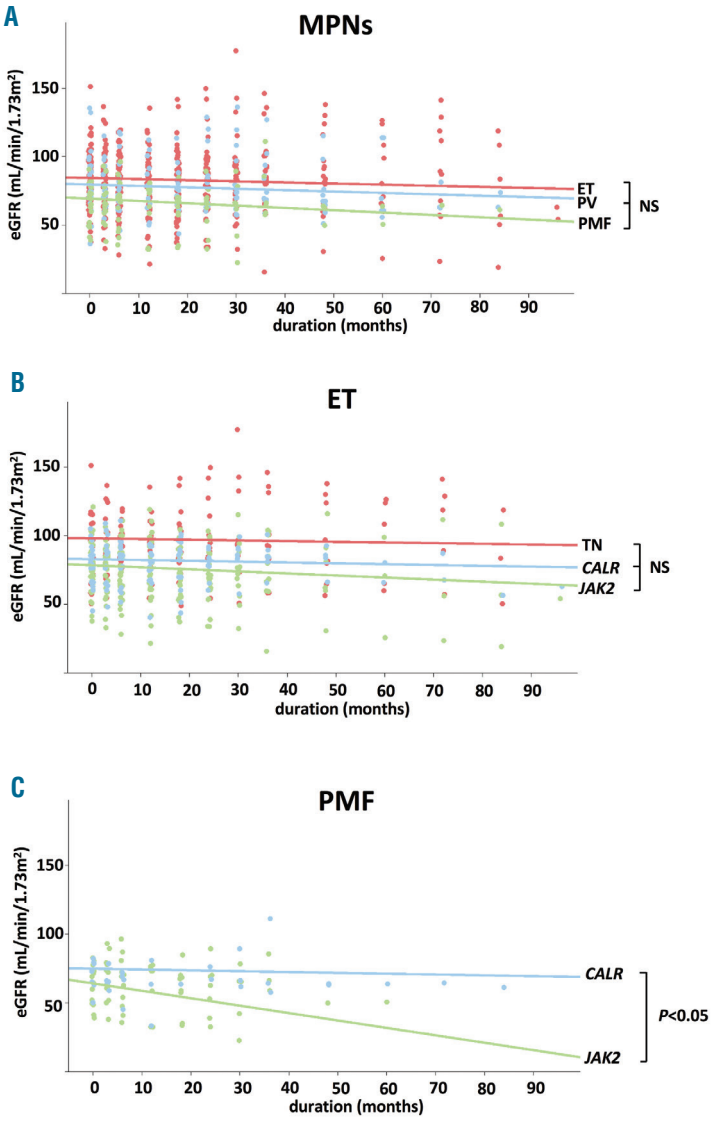

Figure 2. Change in renal function during the disease course in patients with myeloproliferative neoplasms (MPN). (A) Regression lines of estimated glomerular filtration rate (eGFR) values during the disease course in patients with MPN. The rate of change was not significantly different in patients with polycythemia vera (PV, blue), essential thrombocythemia (ET, red), and primary myelofibrosis (PMF, green) (P=0.618). (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) (P=0.344). (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). NS: not significant; n: number.

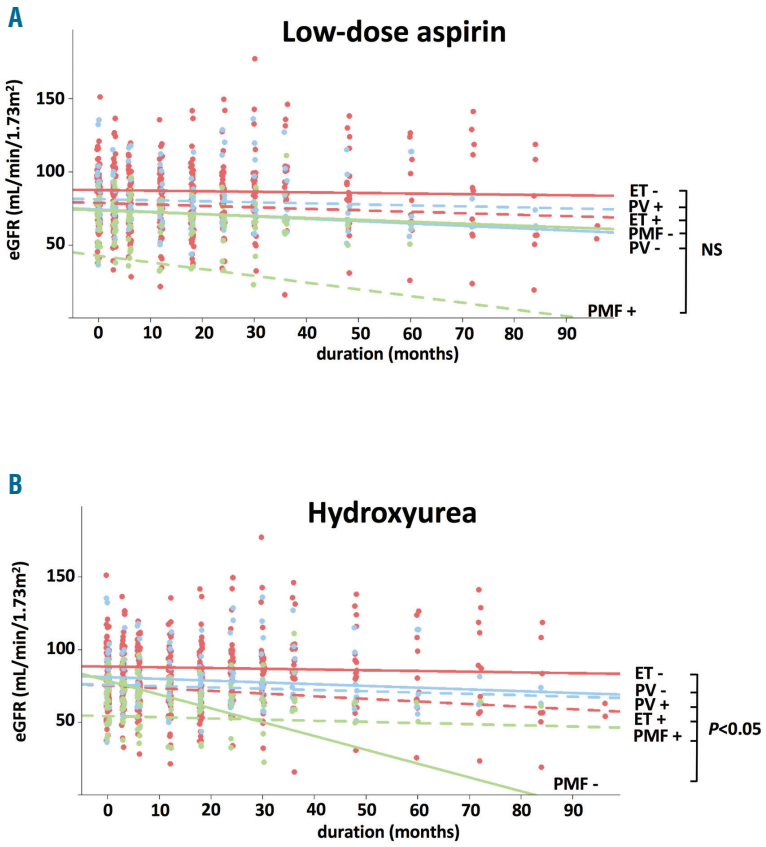

Figure 3. Cytoreductive therapy attenuated the progression of renal dysfunction in patients with primary myelofibrosis (PMF). (A) Regression lines of estimated glomerular filtration rate (eGFR) values during the disease course in patients with different myeloproliferative neoplasms (MPN) subtypes that either received (+) or did not receive (−) low-dose aspirin treatment (P=0.259). (B) Regression lines of eGFR values during the disease course in patients with different MPN subtypes who either received (+) or did not receive (−) hydroxyurea treatment. NS: not significant.

that cytoreductive therapy alleviated renal dysfunction during the disease course in patients with PMF.

Pronounced renal dysfunction in patients with PMF may be a consequence of renal fibrosis manifested as glomerulosclerosis and tubulointerstitial fibrosis, which can be induced by PDGF and TGF-β[9] that are aberrantly increased in PMF.[10] Alternatively, renal extramedullary hematopoiesis associated with PMF[11] may induce renal dysfunction by disorganizing the tissue. Reactive oxygen species (ROS) also promote cytotoxicity and fibrosis in kidneys by generating oxidative stress.[12] Because ROS are elevated in *JAK2*V617F cells,[13] ROS may contribute to the progressive renal dysfunction in PMF patients with that mutation. We examined an association between *JAK2*V617F allelic burden and eGFR in PMF patients at initial diagnosis and found no significant correlation (*Online Supplementary Figure S1*). Regardless of the cause of renal dysfunction in patients with PMF, this pathology was likely prevented by HU treatment that suppresses tumor cell activity.

Finally, to identify the parameters that distinguish individuals with a higher risk of developing renal dysfunction among the patients with MPN, we examined the relationship between laboratory data at initial diagnosis and subsequent change in renal function (*Online Supplementary Table S2*). We found that higher uric acid level was significantly associated with the progression of renal dysfunction during the disease course (P=0.019). The receiver operating characteristic curve analysis determined that 5.8 mg/dL UA was the cut-off value for CKD development (*Online Supplementary Figure S2*).

Because this was a retrospective study, there may be a bias toward PMF patients who had a history of HU treatment in our cohort, which would result in the different rate of progression of renal dysfunction in patient groups treated or non-treated with HU. In fact, PMF patients who received hydroxyurea treatment exhibited significantly higher white blood cell and platelet counts at initial diagnosis compared to those with no history of HU treatment (*data not shown*). However, those parameters did not correlate with the risk of the development of renal dysfunction (*Online Supplementary Table S2*). Thus, these data strongly suggest that HU treatment attenuated the progression of renal dysfunction.

Our study used creatinine level as an important parameter, which, besides renal pathology, can be influenced by various factors, including muscle mass, vigorous exercise, high intake of meat, and plasma volume.[14] Increased plasma volume was linked to splenomegaly in myelofibrosis,[15] suggesting that low eGFR value in PMF patients was due to a high incidence of splenomegaly. However, eGFR value was not significantly different in MPN patients with or without splenomegaly (*Online Supplementary Figure S3*). Nevertheless, a more direct assessment of renal function, e.g. by measuring urinary protein and urine occult blood at initial diagnosis, will be required for a better understanding of the renal dysfunction in patients with MPN. 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. Furthermore, rapid progression of renal dysfunction in patients with MPN was positively associated with uric acid level at initial diagnosis. Because renal dysfunction leads to renal anemia, it is important to prevent the progression of the former in patients with PMF. Our study

strongly suggested that cytoreductive therapy had a suppressive effect on the progression of renal dysfunction in patients with PMF. Based on these findings, we propose that patients with PMF harboring *JAK2*V617F mutation or patients with MPN exhibiting higher uric acid at diagnosis need more frequent checkups for renal function evaluation, and recommend considering the use of cytoreductive therapy to prevent progression of renal dysfunction in such cases.

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