with polycythemia vera in 1995. JAK2 V617F mutation was positive. Myelofibrosis was detected with bone marrow biopsy in 2011. In March 2013, Ruxolitinib was started with 40mg/day. Drug dose was reduced two times due to anemia. The patient was using 20 mg/day Ruxolitinib when viral infection occured. On physical examination there was limitation of the left facial movements and herpetic lesions on the left side of face and left ear. Her hemogram had been as follows; Hb:11.6 g/dl, WBC:18420/µL, Plt:117000/µL, biochemical laboratory results were normal. Electroneuromyography was performed and early period axonal damage findings were detected on left facial nerve. Contrast-enhanced MRI of the ear was normal. Left peripheral facial paralysis due to viral infection was diagnosed. We continued valacyclovir 1000 mg three times in a day and methylprednisolone 80 mg/day and didn't change ruxolitinib dose. In a few week herpetic lesions regressed, facial movements got partially better. We decided to continue antiviral drug as prophylactic treatment. Here we wanted to point out that we should take care about viral infections on people who are using JAK1/JAK2 inhibitor drugs.

Keywords: Ruxolitinib, Ramsay hunt syndrome

PS-052 **Abstract:0165**

CONCOMITANT OCCURRENCE OF DEL5Q AND JAK2V617F MUTATION IN MYELODYSPLASTIC SYNDROME: A NEW CLINICAL ENTITY AND RESPONSE TO LENALIDOMIDE

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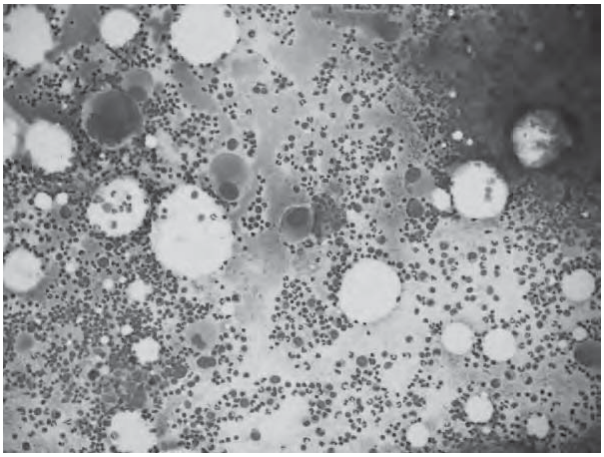
Objectives: The interstitial deletion of the long arm of chromosome 5–del(5q)– is the most frequent cytogenetic abnormality observed in myelodysplastic syndromes (MDS). The concomitant presence of del(5q) and JAK2V617F mutation is very rare, patients have distinct, but still not well-defined, morphological and clinical characteristics of an overlap MDS/MPN disorder. The efficacy of lenalidomide has been established in RBC transfusion dependent patients with IPSS low/intermediate-1 risk del5q MDS.

Case**:** We report a 67-year-old female patient who was admitted to hematology clinics with fatigue. She had macrocytic anemia (Hb 7.7 gr/dl, MCV 100 fL), thrombocytosis (744 x 10^9/L). Her physical examination was unremarkable except paleness. Work up for anemia did not reveal any nutritional deficiency or hemolysis.Physical examination did not reveal any enlargement of liver or spleen. Bone marrow biopsy revealed increased number of dysplastic megakaryocytes and grade II reticulin fibrosis. BCR/ABL1 rearrangement was negative, while the JAK2V617F mutation was identified. The patient was started danasin, methyl prednisolon and aspirin with diagnosis of primary myelofibrosis. However, treatment was discontinued at the end of 16 weeks due to unresponsiveness. Moreover, the patient started RBC transfusions for symptomatic anemia and hydroxycarbamide for increased thrombocytosis of 1.139 x 10^9/L. Her transfusion requirement increased to 2 units RBC every 3 weeks and a transfusional iron overload developed which necessitated addition of deferasirox. The second bone marrow biopsy was moderately hypercellular with E/M= 1:1. Dysplasia was observed in >10% of erythroid and <10% of the myeloid cells. There was marked hyperplasia of morphologically atypical megakaryocytes with monolobated nuclei. Blast cells were <5%, no remarkable increase in reticulin fibers. These findings were consistent with refractory cytopenia with multilineage dysplasia. FISH for del5q(q13q31) revealed positive in 37% of the cells analyzed. Hydroxycarbamide was stopped and the patient was started lenalidomide 10 mg/day for 21 days every 28 days and enoxaparin 0.4 cc/day for thromboprophylaxis. At the end of two cycles, patient's platelet count dropped to normal levels and a mild improvement in transfusion requirement could be noticed. No adverse events were observed except for a mild and transient neutropenia after the first cycle. Patient is currently receiving the third cycle of lenalidomide.

Conclusion: MDS/MPN with concomitant del5q and JAK2V617F is a very rare entity. Awareness to search for both chromosomal abnormality is warranted when common morphological and clinical features are encountered: macrocytic anemia, remarkable thrombocytosis, no splenomegaly, hypercellular marrow with increased number of hypolobated megakaryocytes, no evidence of relevant fibrosis or ring sideroblasts and good response to lenalidomide.

Keywords: del5q, JAK2

Figure 1.



Atypical megakaryocytes with monolobated nuclei

PS-053 **Abstract:0170**

SUCCESSFUL TRANSFECTION OF JAK2V617F LENTIVIRUS

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Objectives: The Janus Kinase 2 gene (*JAK2*) codes for a tyrosine kinase (JAK2) that is associated with the cytoplasmic portion of a variety of transmembrane cytokine

and growth factor receptors important for signal transduction in hematopoietic cells. Signaling via JAK2 activation leads to cell growth and differentiation. *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs) frequently harbor an acquired single nucleotide mutation in*JAK2* characterized as Val617Phe (V617F) in the protein. This mutation is identified overall in approximately two-thirds of all MPNs. Lentiviral vectors are commonly used as gene delivery vehicles. In this study, it is aimed to produce JAK2V617F virus.

Methods: *E. coli* DH5α strain was transformed with lentiviral transfer vectors (LTVs) which have the wild type (wt) *JAK2* or *JAK2* with V617F mutation insert, which also have *GFP* (Green Florescent Protein) gene. LTV that carry only *GFP* insert, and two additional plasmids (pCl-VSVG, pCPRDEnv) required for viral packaging were also transferred into the bacteria separately by electroporation. Some colonies of transformed bacteria were selected and produced in LB medium. Plasmid DNAs were isolated from bacterial cells and subjected to restriction digestion to confirm correctness of the plasmids. Next, 293T (Human Embryonic Kidney) endothelial cells were transfected with the isolated plasmid vectors using lipofectamine. Transfection efficiency was evaluated by flow cytometry analysis.

Results: The plasmid DNAs (JAK2wt-GFP, JAK2V617F-GFP, GFP, pCl-VSVG, pCPRDEnv) were confirmed by digestion with proper restriction enzymes on the agarose gel electrophoresis. 60% and/or higher transfection efficiency was detected in the each cell line with JAK2wt-GFP, JAK2V617F-GFP and only GFP by flow cytometry analysis. Viral particles were harvested from the supernatant of these cell lines by ultracentrifugation. The green color of GFP proteins were also detected under the light microscope.

Conclusion: Lentiviral vector plasmids were cloned in *E. coli* cells and lentiviral particles were produced in 293T cells successfully. This method provides variety of opportunities for understanding the effects of JAK2V617F mutation on individual cell types including hematopoietic cell and stem cell. In conclusion, our study could provide an understanding of the JAK2V617F mutation effects on the cells in the context of MPNs.

Keywords: JAK2V617F mutation, Lentivirus

PS-054 **Abstract:0250**

GENE EXPRESSION ANALYSIS OF JAK2V617F MUTATION IN POLYCYTHEMIA VERA

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Objectives: Detection of mutations activating JAK-STAT pathway identified tyrosine kinase activation causing Myeloproliferative Neoplasm (MPN) pathogenesis. JAK2V617F mutation is seen in 95% of Polycythemia Vera (PV) patients. JAK activation stimulates cellular events like cell differentiation, proliferation and apoptosis. JAK-STAT pathway gene expression profiling is critical for the gene analysis. In this study it is aimed to understand the effects of genes in JAK-STAT signaling, apoptosis, immune response, cell growth and maintenance by JAK-STAT PCR array.

Methods: Gene profiling was started with 3 different PV patients that were wild-type, heterozygote and homozygote mutant for JAK2V617F mutation. Mononuclear cells (MNC) for patients were taken from liquid nitrogen and thawed. After RNA isolation, cDNA synthesis was performed prior to real-time PCR. SYBR Green based detection was accomplished. 96-well format PCR array made it possible to profile 84 genes utilizing the threshold cycle values from the light cycler, fold changes were calculated for each genes comparing with control.

Results: Expression level of housekeeping genes that were ACTB, B2M, GAPDH, HPRT1 and RPLP0 examined and quality was checked for PCR Array reproducibility, RT efficiency and gDNA contamination. To obtain fold change of gene expression for homozygote and heterozygote mutant, wild type patient was used as a control. CDKN1A was 4 fold downregulated in heterozygote than homozygote mutant and FAS was about 2 fold downregulated in heterozygote than homozygote mutant patient. OSM was 1.5 fold upregulated in homozygote mutant but, downregulated in heterozygote patient. OSM encodes a growth regulator that inhibits the proliferation of a number of tumor cell lines. Protein encoded by CDKN1A functions as a regulator of cell cycle progression at G1 and protein encoded by FAS has central role in the physiological regulation of apoptosis. It was shown that activating JAK mutations are major molecular events in human hematological malignancies. JAK2V617F mutation caused a reduction in the gene expression level in the JAK-STAT pathway.

Conclusion: The study was carried out with a limited number of patients but it is important for the understanding of the effects of the mutation on the genes and gene expression profiling technology for analyzing the panel of genes in signal transduction pathways, biological processes and disease related gene networks. Further studies are going to be advanced by working with more patients.

Keywords: JAK-STAT pathway, Polycythemia Vera

PS-055 **Abstract:0258**

IMPORTANCE OF MPL MUTATION ANALYSIS: AN ADVANCED AGE FEMALE MPN PATIENT

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Objective: BCR-ABL1 negative Myeloproliferative neoplasms (MPN's) includes polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET). Janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL) are the molecular markers used for the molecular diagnosis of MPN. However, the CALR and MPL mutations have not been classified yet in the WHO diagnostic criteria for BCR-ABL1 negative MPN's. Here we discuss the importance of MPL in this group of diseases over an example.

Method: Karyotype analysis and FISH (-5/5q-, 20q-, +8, -7/7q-) were performed on cultured bone marrow cells. JAK2 V617F mutation analysis and BCR-ABL1