prepared plasma sample from *JAK2V617F*-positive blood can be interpreted falsely as "negative." In addition, the interval between drawing of blood and purification of granulocytes must be kept below 48 hours to avoid significant decrease in the apparent *JAK2V617F* allele burden.

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Response

Plasma and JAK2 mutation

Salama et al reported on the stability of *JAK2V617F* DNA (17 patients) and RNA (1 patient) in whole blood specimens from subjects with V617F-positive myeloproliferative neoplasms, specifically comparing the percentage of V617F relative to wild-type sequence in granulocytes and plasma at several times after collection of whole blood. They indicate that the percentage of *V617F* DNA and RNA changes during storage of whole blood samples, decreasing in cells (at 0, 4, and 11 days) and increasing in plasma (at 2, 4, 7, 9, and 11 days) with time, after sample collection.

Unfortunately, interpretation of their findings may be hindered by the use of Histopaque 1077 (Sigma-Aldrich) for granulocyte isolation. Because this gradient-based method is intended to isolate mononuclear cells rather than granulocytes,[1] we cannot be certain that their cell-based measurements in fact represent granulocyte DNA or mixture of lymphocytes and immature granulocytes and monocytes. Moreover, plasma separated by gradient is not the same as plasma separated by simple centrifugation. The effects of the gradient circulating RNA is not known and may have the potential of trapping RNA.

Methodological complications aside, their conclusion that "[plasma] should not be used in clinical practice to quantify *JAK2V617F* or address zygosity" appears to be incomplete. That is, while the percentage of *JAK2V617F* allele increased over time in plasma, it decreased in "granulocytes" at what appeared to be an even higher rate. Thus, a more appropriate conclusion would have been that neither granulocyte nor plasma quantification of *JAK2* mutation is suitable for clinical testing of samples stored long term at room temperature.

In fact, these results are not surprising; for most analytes, biological samples typically should not be kept at room temperature for 4 days or longer prior to assay. It is expected that cells will lyse in a blood sample after 2 days. Lysis of leukemic cells is even more likely if the patient has been receiving therapy. Salama et al did not report the treatment status of their patients. Thus, the clinical relevance of most of the time points used in Salama et al's study is unclear. In addition, the significant reduction in the percentage of *V617F* in the granulocytes is contradictory to the current belief that all these cells are leukemic and carry the V617F mutation, and the percentage of mutant should not change because some of these cells are being lysed. This differential change in the ratio of the mutant level most likely reflects that they are analyzing mononuclear cells rather than granulocytes, or indicates issues with the formula for calculating the percentage of the mutant DNA. A previous study by authors using this same quantitative PCR assay also showed a lack of concordance with the literature.[2]

In conclusion, testing of plasma or granulocytes for the purpose of measuring tumor load should be performed as soon as possible after collection of whole blood samples. Alternatively, plasma may be separated from cells promptly for further testing, particularly if the patient is receiving therapy. Furthermore, well-controlled studies are needed to establish the effects of other factors, such as fever, infection, and kidney problems, that may influence circulating nucleic acid.

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