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Monitoring de l'immunosuppression par les thiopurines dans les maladies inflammatoires chroniques de l'intestin par le suivi de la macrocytose

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Mean Corpuscular Volume (MCV) to monitor azathioprine and 6-mercaptopurine treatment in Inflammatory Bowel Disease (IBD) patients.

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Abstract

Background: Erythrocyte MCV might be used as an inexpensive marker to predict and optimize the efficacy and tolerability of thiopurine therapy in IBD patients.

Aim and methods: This retrospective observational study aimed to assess the monitoring performances of MCV in patients under 3 months or more thiopurine treatment followed up in the Swiss IBD Cohort Study. All available MCV, white blood cells (WBC) and 6 thioguanine nucleotide (6TGN) measurements, among others, were recorded. An IBD "flare" was defined as a composite outcome encompassing treatment change, colonoscopy, histology, CT scan or MRI reports showing active IBD lesions, occurrence of intestinal surgery and IBD-related hospitalisations. Whether MCV measurements predicted efficacy of thiopurine treatment was investigated by assessing the statistical association between the occurrence of IBD "flares", and the current or recent MCV values, taking into account the patient clustering and longitudinal aspect of data.

Results: 140 patients (77 women), mean age 38 years (17-74), 104 diagnosed with Crohn's disease, 36 with ulcerative colitis, mean disease duration 8 years (0.25-36), receiving either azathioprine (n=125) or 6-mercaptopurine (n=15) were included, most of them over 3-year follow up.

Thiopurines increased mean patient MCV by an average 5.8±5.2 fL, while patientsfluctuated by ±4.3 fL around their individual mean ($p<0.001$). They decreased WBC by an average of 2.4+/- 2.6 G/L ($p<0.001$).

Significant associations were observed between the probability of flare occurrence and low current MVC ($p=0.017$) or high current WBC ($p=0.009$) and, with a relative risk of 3.7% for every fL of MCV decrease or 8% for every G/L of WBC increase. Both markers revealed some memory effect.

Despite this, the performance of MCV and WBC to predict IBD "flare" remained rather limited, as it is less accurate than the 6-TGN-level , although only determined in a subgroup of patients in this study.

Conclusion MCV and WBC deserve to be observed to check and monitor therapeutic exposure to thiopurine agents in IBD patients. Unfortunately, their predictive performance precludes their privileged use for optimization of therapy. Further prospective studies should suitably include the systematic measurement of metabolite concentration.

Key words : MCV, Thiopurine, IBD, Monitoring, Flare

Background

About 12 000 patients are affected by inflammatory bowel disease (IBD: Crohn's disease, ulcerative colitis, indeterminate colitis) in Switzerland (1). Among them, approximately 45% receive thiopurines as immunosuppressant treatment (azathioprine 40%, 6-mercaptopurine 5%).

As demonstrated in several controlled trials versus placebo (2; 3; 4) and meta-analyses (5; 6; 7), thiopurines (azathioprine and 6-mercaptopurine) are effective as steroid sparing drugs and for maintenance of remission in IBD (40; 41; 42; 43). Alleviation of clinical symptoms and signs of inflammation as well as, more recently, mucosal healing, represent the main objectives of the treatment (44).

Azathioprine (AZA) and its metabolite 6-mercaptopurine (6-MP) are purine base analogues that inhibit biosynthesis and incorporation of purine nucleotides in cells during mitosis (8). Consequently, they interfere with the maturation of not only immunocompetent cells, but also erythroid precursors (7), thus increasing red blood cells MCV (14; 15; 16). Similarly, they affect myeloid precursors, making neutropenia a well-known dose-dependent effect of thiopurine therapy.

MCV is a measurement of the average red blood cell size and is calculated by dividing the haematocrit by the red blood cell count (16). The widely used automated blood cell counters make such marker readily available and relatively inexpensive.

Thiopurines are prodrugs given on the long term with a complex metabolism characterized by large inter-individual variability (9), including variable bioavailability (10,11,14). Thiopurine methyltransferase (TPMT), the cytosolic enzyme that metabolises azathioprine *in vivo*, exhibits genetic polymorphism. The risk of azathioprine induced myelosuppression corrélâtes with intermediate or low TPMT activity. As in most long-term therapies, patient adherence represents a potential issue. Such treatments ideally require methods for control of efficacy. Chronic care could potentially be improved, and possibly at reduced cost, by implementing rational monitoring strategies (12).

Recently, a study established that MCV in patients under effective azathioprine treatment was significantly greater than in those for whom the treatment was rated as ineffective. The authors suggested that measurements of variation in MCV values could serve as a guide to adjust the dose in patients with Crohn's disease (15). In the past, investigators had already reported that an increase in MCV was commonly observed during thiopurine therapy, and that the change in the MCV may represent a helpful response marker in patients during thiopurine therapy for Crohn's disease (11). Two further studies confirm that patients experiencing a clinical response to treatment with thiopurines have greater MCV increases than non-responders, suggesting that a change in MCV anticipates the therapeutic response in patients with Crohn's disease (10), and that MCV might be used as a cost effective marker to guide 6-mercaptopurine therapy in IBD (13).

Erythrocyte MCV, known to increase under thiopurines, might thus be used as an inexpensive monitoring marker to predict and optimize the efficacy and tolerability of thiopurine therapy in IBD patients.

The present study is a retrospective observational analysis aimed to assess the potential monitoring performances of MCV and WBC in patients under azathioprine or 6-mercaptopurine treatment followed up by the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS) in three participating centres (University Hospital Centre of Lausanne, Clinique la Source in Lausanne, and Hôpital Neuchâtelois).

Material and Methods

Patients

The Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS) provided the population sample. The SIBDCS, running from 2005, is a national disease-oriented prospective cohort that brings together 6 main centres (Basel, Bern, Geneva, Lausanne, Zurich, St Gallen), hospital centres and gastroenterologists in private practice who agreed to participate. In October 2011, around 2200 patients gave their consent to participate in this study. Inclusion criteria target IBD patient with a diagnosis established at least 4 months

prior to inclusion or having had at least one recurrence of their symptoms, permanently residing in Switzerland or having contracted Swiss health insurance, and being treated on a regular basis in Switzerland. Participation is not restricted by age or sex; it is voluntary and relying on individual motivation. Diagnosis, based on Lennard–Jones criteria (36), can be confirmed by radiological, endoscopic and histological findings or surgery (37). Our study specifically included IBD patients (Crohn's disease or ulcerative colitis) receiving prolonged (>3 months) treatment of azathioprine or 6-mercaptopurine, for whom a medical record could be consulted from either hospitals or physicians in our regional area.

Treatments

All Azathioprine and/or 6-mercaptopurine dosages were collected retrospectively from patient charts. Treatment allocation, doses, follow up and co-medication were defined by physicians in charge of the patients. We had no information on treatment compliance.

Observations

Clinical and biological data routinely recorded into the SIBDCS data base are, among others, : gender, age, weight, diagnosis, date of diagnosis, severity of disease as defined by the Crohn's disease activity index (CDAI) for Crohn's disease (17) and by the Modified Truelove & Witts activity index (MTWAI) for ulcerative colitis (18), clinical course (phenotype according to Montreal classification for state of disease), treatment (dose, duration and evolution), therapy adverse effects, past therapy, supplementation therapy (iron, Vit.B12, folic acid), number of IBD flares, complications and/or hospitalisations due to the IBD. The following laboratory parameters are recorded in the database on a yearly basis: leukocytes count, haemoglobin (Hb), haematocrit (Ht), C-reactive protein (CRP), erythrocytes sedimentation rate ESR, ferritin, vitamin B12, folic acid, alkaline phosphatase and albumin.

In addition, the medical records of the patients included were reviewed manually to record the following supplemental data, when available: MCV, azathioprine and/or 6-mercaptopurine dose, haemoglobin count, haematocrit, erythrocytes, leukocytes count (WBC), neutrophils and platelets counts, mean platelet volume, ferritin, Vitamin B12, folic acid, C-reactive protein, erythrocyte sedimentation rate, 6-thioguanine nucleotide (6-TGN)

and 6-methylmercaptopurine (6-MMP) blood concentrations (these thiopurine metabolites were considered to have been determined at the end of a dosage interval, in the absence of precise information regarding last dose intake time). Different laboratories in Switzerland measured MCV and we have no precise information on the method of measurement or on the laboratories validation. However, MCV is mostly measured using volume-sensitive automated blood cell counters by optical means.

Blood tests were collected through medical records with one-week interval or more from the beginning of the thiopurine treatment. If available the two last blood tests before treatment were collected, respecting also a one-week interval.

Medical treatment changes, colonoscopy, histology, CT scan and MRI reports, occurrence of intestinal surgery, hospitalisation due to the IBD, occurrence of extra-intestinal symptoms (uveitis, erythema nodosum, pyoderma gangrenosum, severe arthritis), presence of stenosis and /or dilatation were also timely recorded.

Composite outcome

An IBD event or "*flare"* was defined as a composite outcome encompassing any introduction of new IBD medical treatment (anti-TNF therapy, methotrexate, steroid, mesalazine), active IBD lesions shown on colonoscopy, histology, CT scan, MRI report, occurrence of intestinal surgery and hospitalisation related to IBD, occurrence of stenosis and/or dilatation, and a CDAI greater then 150 or a MTWAI greater or equal to 10.

Statistical analysis

All collected variables were subjected to descriptive analysis. Results were expressed as mean or median (± standard deviation or range) for numerical variables and as±

percentages for qualitative variables. A p-value less than 0.05 was considered statistically significant during comparisons with standard tests (T-test, linear correlation).

The effects of time and thiopurine dose on MCV and WBC values were explored using multilevel mixed effect linear regression analysis (accounting for hierarchical random patient effect). A variogram analysis was also applied to the time sequences of MCV and

WBC, to observe the time-related change in variance between initial on-treatment values and subsequent values; assuming patient-specific linear long-term trends for these markers, it is appropriate to fit the variograms with a quadratic model for time, again using multilevel mixed effect linear regression (39). Finally, in a nested case-control inspired approach, the effect of MCV, WBC and further factors on the probability of IBD event occurrence was explored using multilevel (hierarchical) mixed effect logistic regression analysis (accounting for random patient susceptibility). Calculations were performed using the Excel (Microsoft, 2011) and STATA software (v. 11, StataCorp, College-Station TX, 2007).

Results

Study population and treatments

We were able to include 140 patients (77 women), with a mean age of 38 years (range: 17-74), 104 diagnosed with Crohn's disease and 36 with ulcerative colitis, with a mean disease duration 8 years (0.25-36), currently receiving either azathioprine (n=125) or 6-mercaptopurine (n=15), most of them with 3-year follow up. We recorded a total of 1594 MCV determinations and 256 "flares" in these patients; pre-treatment MCV values were available in only 61 patients. Among patients under azathioprine, 54 had 6-TG and 6-MMP blood determinations. Mean daily azathioprine dosage was 135 mg (SD: ± 48 mg). Mean daily 6-mercaptopurine dosage was 72 mg (+/- 25 mg).

Number of patients	140
Women : Men	55% : 45%
Mean age (range)	38 (17 – 74)
Crohn's disease : Ulcerative colitis	75% : 25%
Mean disease duration in years (range)	8 (0.25 – 36)
Azathioprine : 6-mercaptopurine	90% : 10%
Number of MCV determinations per patient (mean ± SD)	11.6 ± 7.6 (0 – 39)
Number of WBC determinations per patient	11.6 ± 7.5(0 – 39)
Daily azathioprine dose (mean ± SD)	135 ± 48 mg
Daily 6-mercaptopurine dose	72 ± 25 mg
Number of "flares" reported per patient	1.8 ± 2.2 (0 – 12)

Table 1 : Characteristics of study population, treatments and outcomes

Factors affecting the occurrence of "flares"

Time related evolution of "flare" occurrence

Under the hypothesis of a similar intensity of follow up between studied patients, we observed a patient factor that explained 24% of the variability in "flare" occurrence ($p<0.001$).

There seemed to be no significant time trend increase in the occurrence of "flares", with a +3%/year slope ($p=0.43$)

Influence of current and past therapy on the occurrence of "flares"

The occurrence of events was different under AZA and 6-MP, the latter being associated with a greater risk of "flare" (Relative risk : 1.5, p=0.05); this is however likely to reflect therapeutic decisions taken in patients with frequent recurrences, rather than a proper characteristic of the drugs.

We observed no association between the daily dosage of AZA and 6-MP and the frequency of "flare" occurrence (p=0.17, resp. 0.74). If we combine the current AZA daily dose with the mean dose over one to twelve month before, both it then appears significantly related to flare occurrence, with opposite effects. This might well result from therapeutic decisions taken in patients with frequent recurrences.

We did not find any influence of the 6-MP doses history on flares frequency.

Time related evolution of azathioprine and 6-mercaptopurine doses

We did not observe any clear time trend in thiopurine daily dosages, the slight increase observed in mean azathioprine or 6-mercaptopurine (Azathioprine 2.7 mg/year, 6-mercaptopurine 1.9 mg/year) did not reach significance (p= 0.08, resp. 0.11). Neither did we observe any significant time trend in metabolites concentration monitoring. On the other hand, we did not observe any effect of a change in dosage during the past months.

Factors affecting MCV and WBC

Global effect of the treatment on MCV and WBC

Thiopurines increased mean MCV by an average 5.8±5.3 fL in patients providing pre-treatment values, while patients fluctuate by ±4.3 fL around their individual mean (p<0.001 for patient clustering). A patient effect thus explains 59% of the variance (however, as the values are differences with pre-treatment baseline, up to 50% may be due to variability from the baseline value, thus leaving a mere 9% that accounts for individual sensitivity).

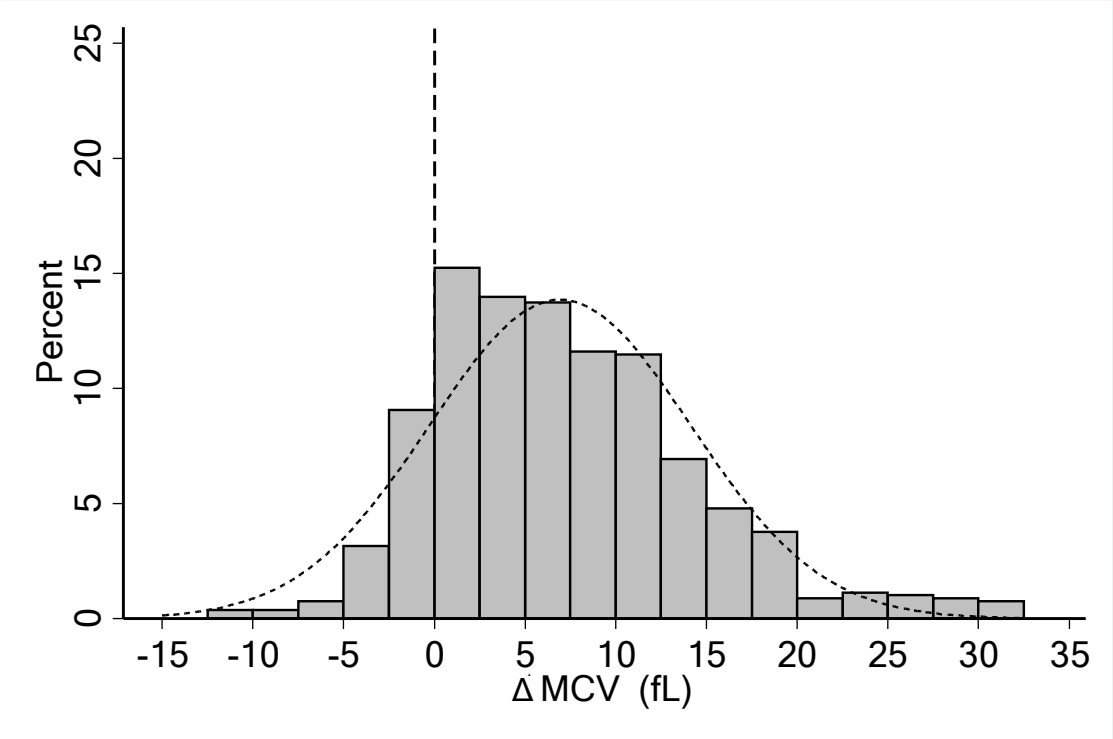


Figure 1: Distribution of MCV changes in fL from pre-treatment baseline (ΔMCV)

The same approach applied to WBC also revealed a significant patient effect ($p<0.001$), the patients decreasing by an average of 2.4 ± 2.6 G/L under treatment from their pre-treatment value, while fluctuating by 3.2 G/l around their individual mean. The patient effect explains 50% of the variance (which presumably results from variability in baseline levels, thus leaving no variability in individual sensitivity).

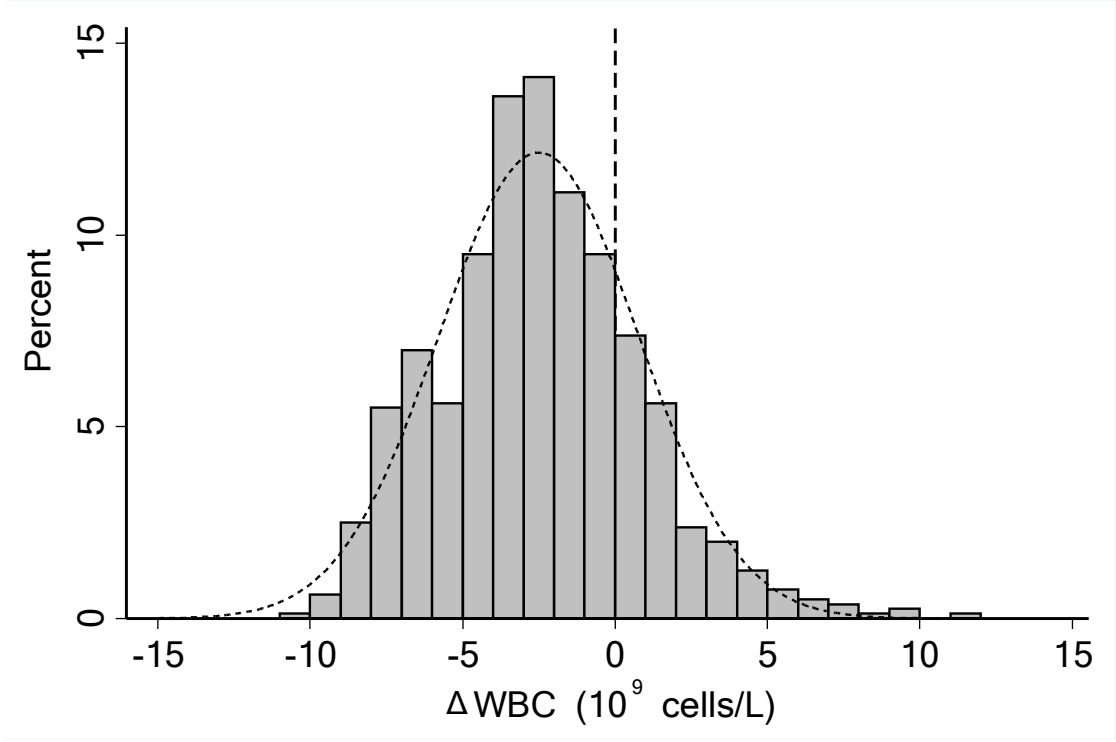


Figure 2: Distribution of WBC changes (in 10^9/L) from pre-treatment baseline (ΔWBC)

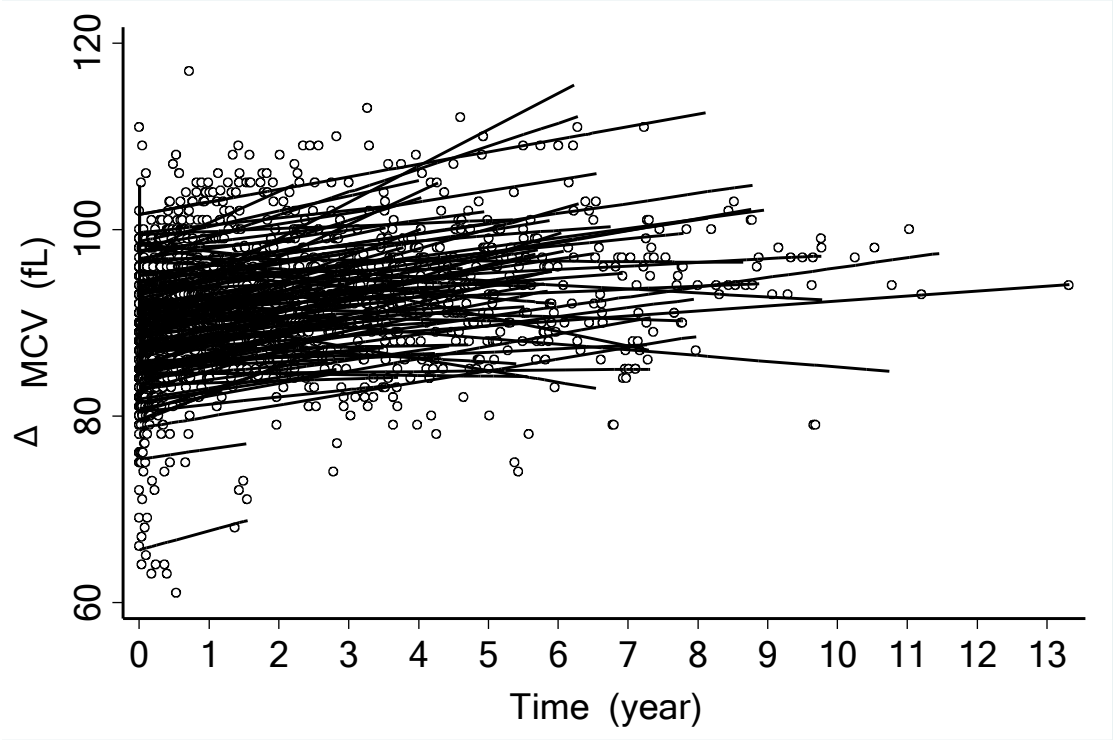
Influence of the thiopurine type (AZA or 6-MP) on MCV and WBC

6-MP appeared to increase MCV by an additional 3.6 fL compared to AZA ($p<0.001$).

Conversely, we did not observe any influence of the thiopurine type on WBC ($p=0.88$).

Influence of treatment duration on MCV and WBC

Independently of drug dosage, which did not show any time-related trend, MCV tended to increase along treatment duration (slope: 1.2 ± 1.3 fL/year, $p<0.001$), with a significant heterogeneity in individual slopes and intercepts ($p<0.001$). A similar association was also observed with the WBC (negative slope: -0.25 +/- 0.19 G/L/year, $p<0.001$), however without significant slope heterogeneity ($p=0.09$).



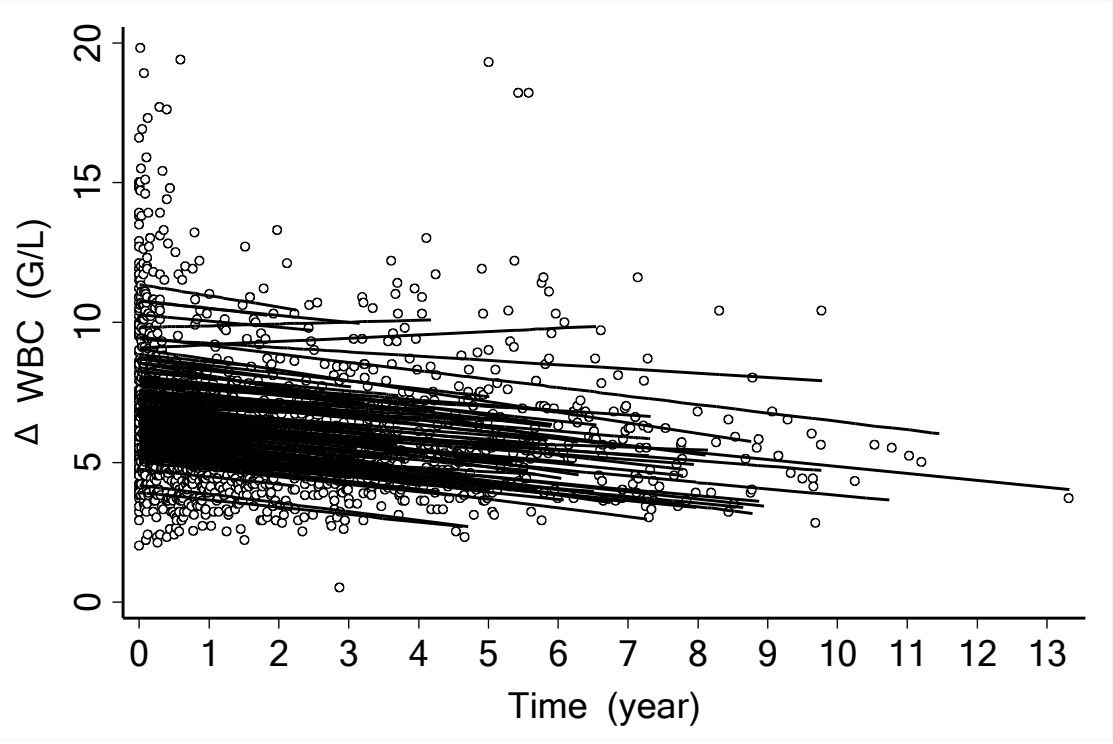


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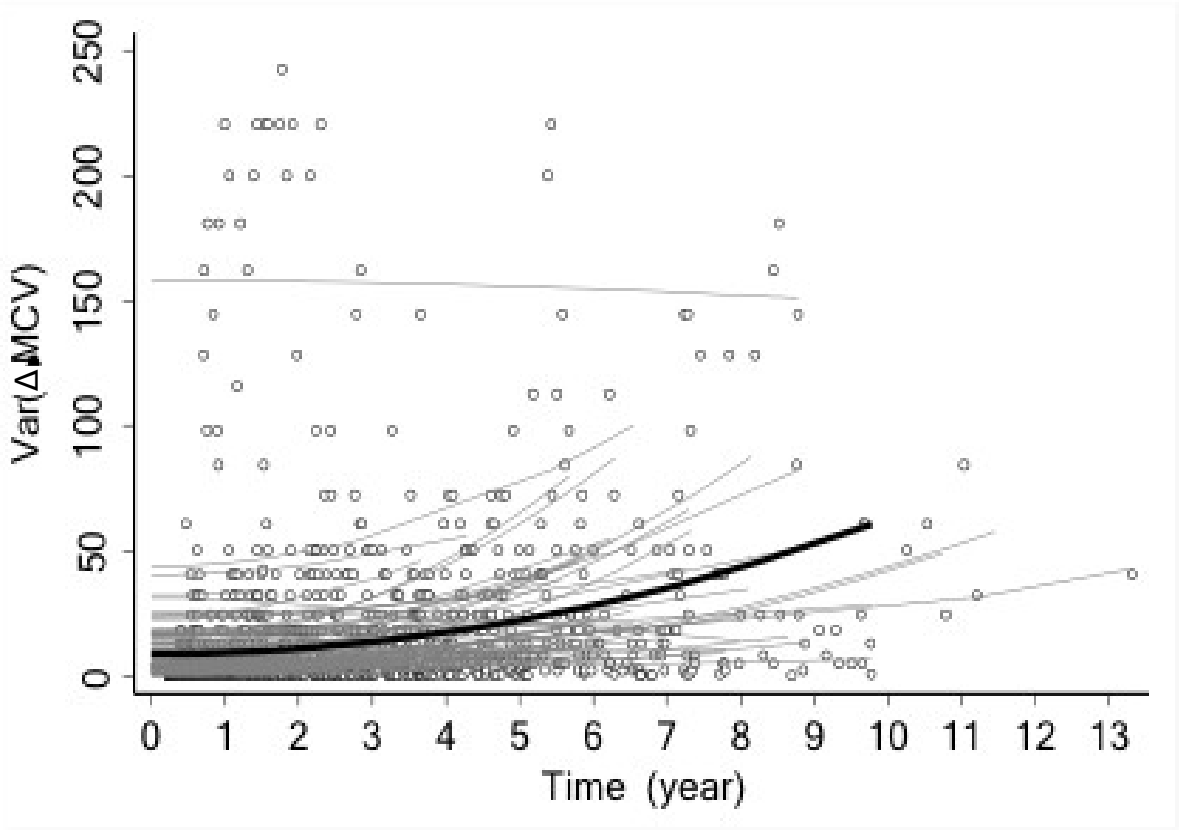
Figure 3: Individual MCV and WBC values and patient specific linear trends

MCV and WBC variogram

We estimated the time-related increase in variance between the first ongoing treatment MCV or WBC value and the subsequent MCV, WBC values measured during treatment.

For MCV, the instantaneous (extrapolated to time zero) variance was 2.9 ± 4.2 fL. Only after 4 years did the increase in variance reach a magnitude similar to the instantaneous variance. This means that roughly 4 years would have to be waited before remeasuring MCV to have a reasonable chance (50%) to impute a change to a real drift of MCV value in the patient.

For WBC we could not find any time related tendency, there was no drift of the variogram along time, in accordance with the absence of heterogeneity in patient trajectories.



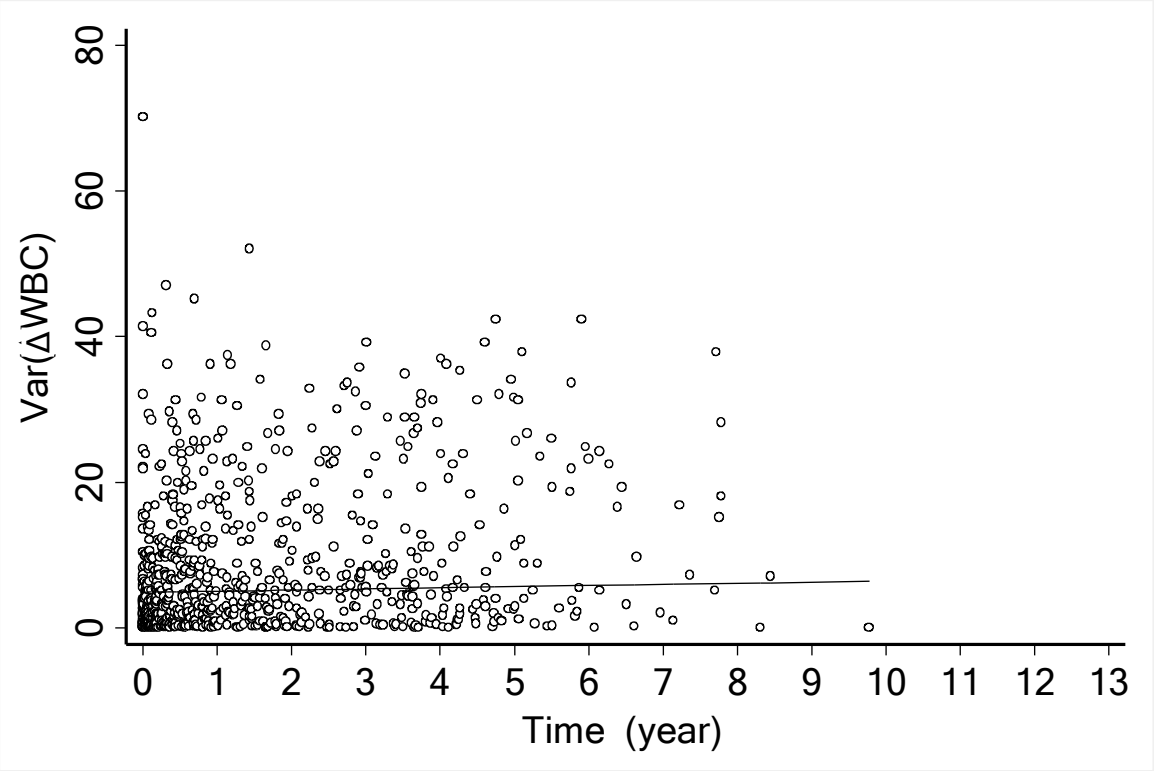


Figure 4 : MCV and WBC variograms along time; variances in Δ MCV are appropriately fitted by hierarchical quadratic regression, while variances in Δ WBC show no significant time related trend

Influence of current AZA doses on MCV and WBC

Patients under AZA showed a significant association between their current AZA dosage and MCV, amounting to 0.044 fL per mg/day ($p<0.001$), while accounting for patient effect on MCV level. The same association was observed for WBC, showing a decrease by 0.017 G/L per mg/day ($p<0.001$). We found the same association looking either at raw values or at changes of MCV and WBC from the corresponding baseline pre-treatment value. A random intercept linear model fitted the data adequately, accounting for patient effect without indication of variability in individual dose-response slopes.

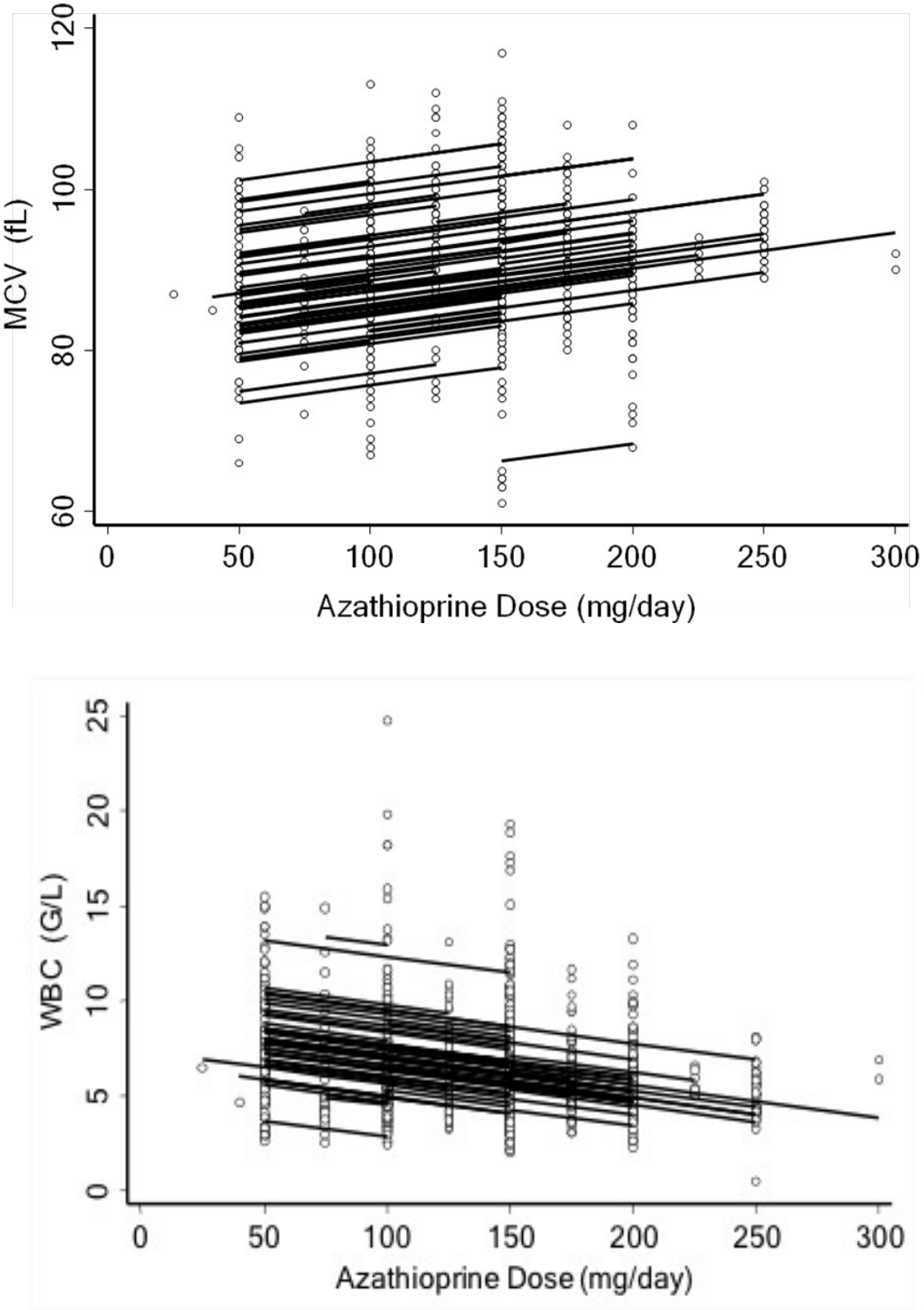
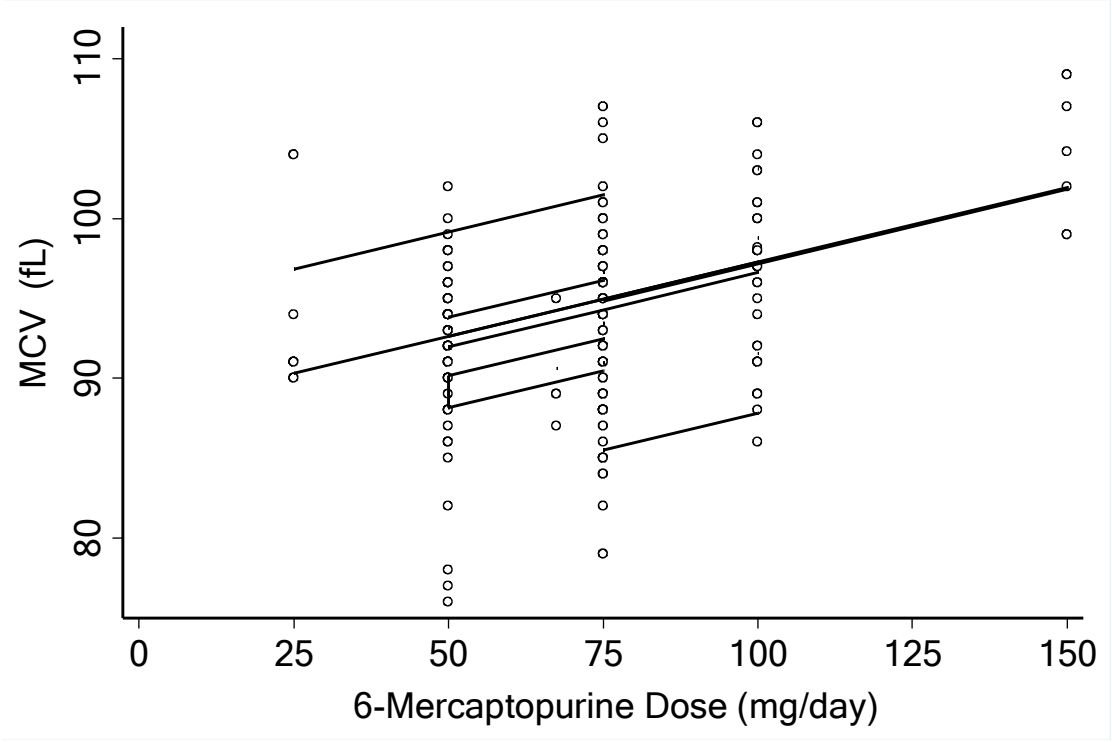


Figure 5: Association between AZA dose and MCV and WBC (individual dose-response lines are drawn only for patients experiencing dosage changes)

Influence of current 6-MP doses on MCV and WBC

Patients under 6-MP showed as well a significant association between current 6-MP dosage and MCV, of 0.093 fL per mg/day ($p<0.001$). Here again, we found the same association looking either at raw values or at changes of MCV compared to the basal pre-treatment value.

Patients under 6-MP, showed a similar but non-significant association between actual 6-MP doses and WBC, possibly due to the small number of individuals.



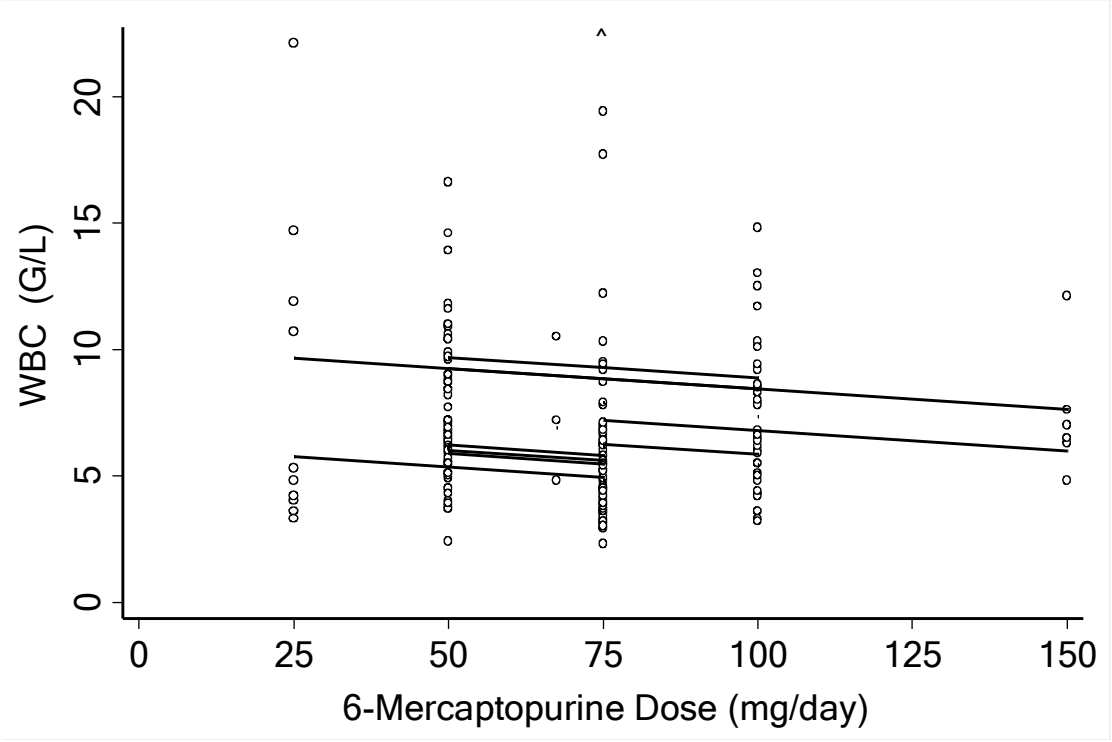


Figure 6: Association between 6-MP doses and MCV and WBC (individual dose-response lines are drawn only for patients experiencing dosage changes)

Influence of the history of AZA and 6-MP doses on MCV and WBC

On analysing the sequence of integrated AZA doses over the past months, we observed an association with MCV and WBC ($p<0.001$) increasing up to 4 month before determination. A similar association was observed for the change of MCV and WBC from the baseline value.

On combining current AZA doses with the cumulated dose over one or several past months, we observed a stronger association with the integrated dose ($p<0.001$), with a corrective influence of the current dosage.

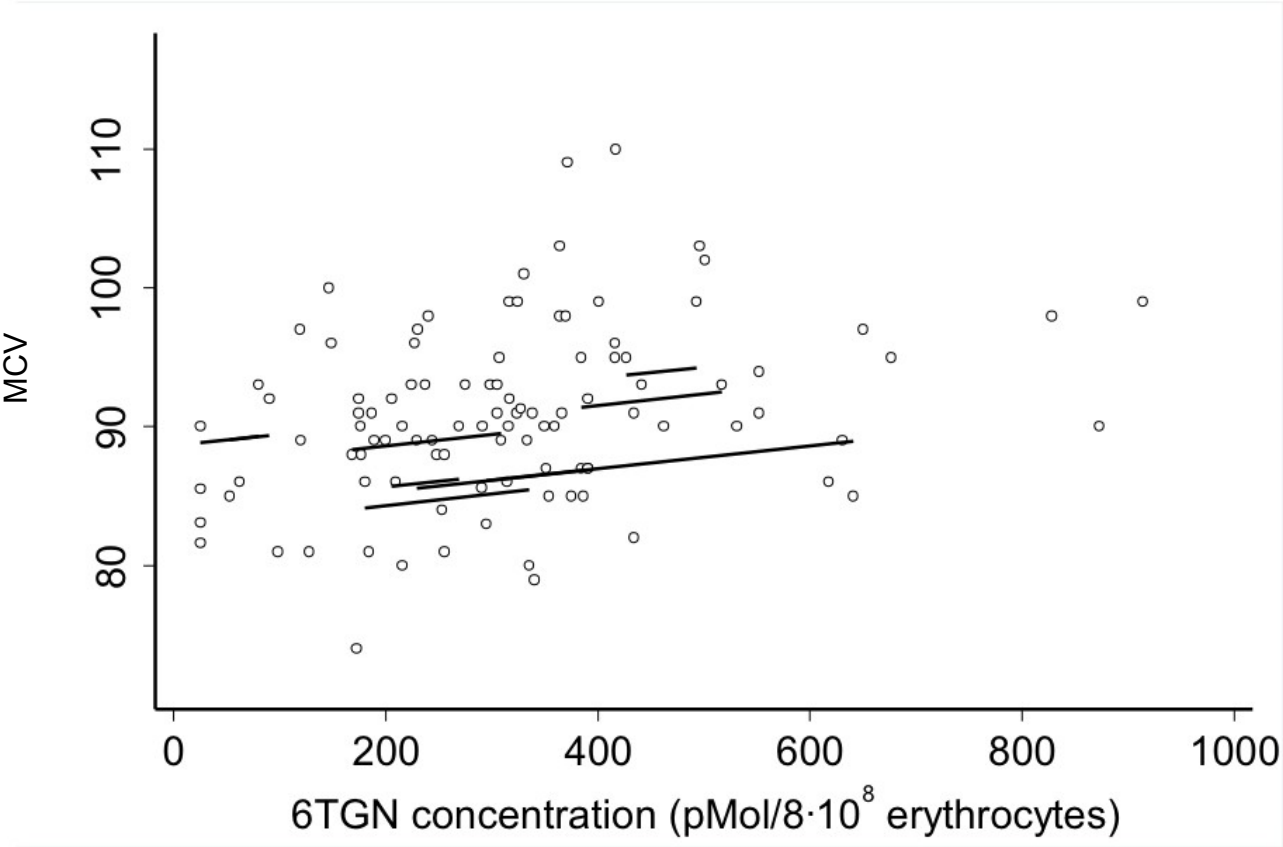
Similarly, the sequence of integrated 6MP doses over the past months revealed an association with MCV ($p<0.001$), increasing up to 2 months, and with WBC ($p=0.037$), detectable up to 6 months before determination. Combining current 6MP doses with the mean dose over one or several past months revealed a stronger association with the

integrated dose (MCV p<0.001 ; WBC p=0.092), without any more influence of the current dose.

Influence of the concentrations of 6-thioguanine nucleotide (6TGN) and 6-methylmercaptopurine (6MMP) on MCV and WBC

In the subset of 102 patients having provided thiopurine metabolites concentration results under AZA, MCV appeared to be correlated with 6TGN concentrations, with a slope of 0.0082 fL per pmol/8·10^8 erythrocytes (p=0.016, hierarchical linear regression). This association was stronger than with AZA dose in those patients (p=0.017). MCV was not correlated with 6MMP. Conversely, WBC values revealed no correlation with 6TGN concentrations, but were slightly associated with 6MMP concentrations, however with a rather flat slope of -0.00012 G/L per pmol/8·10^8 erythrocytes (p=0.04).

There were not enough concentration measures in patients receiving 6MP to draw a conclusion.



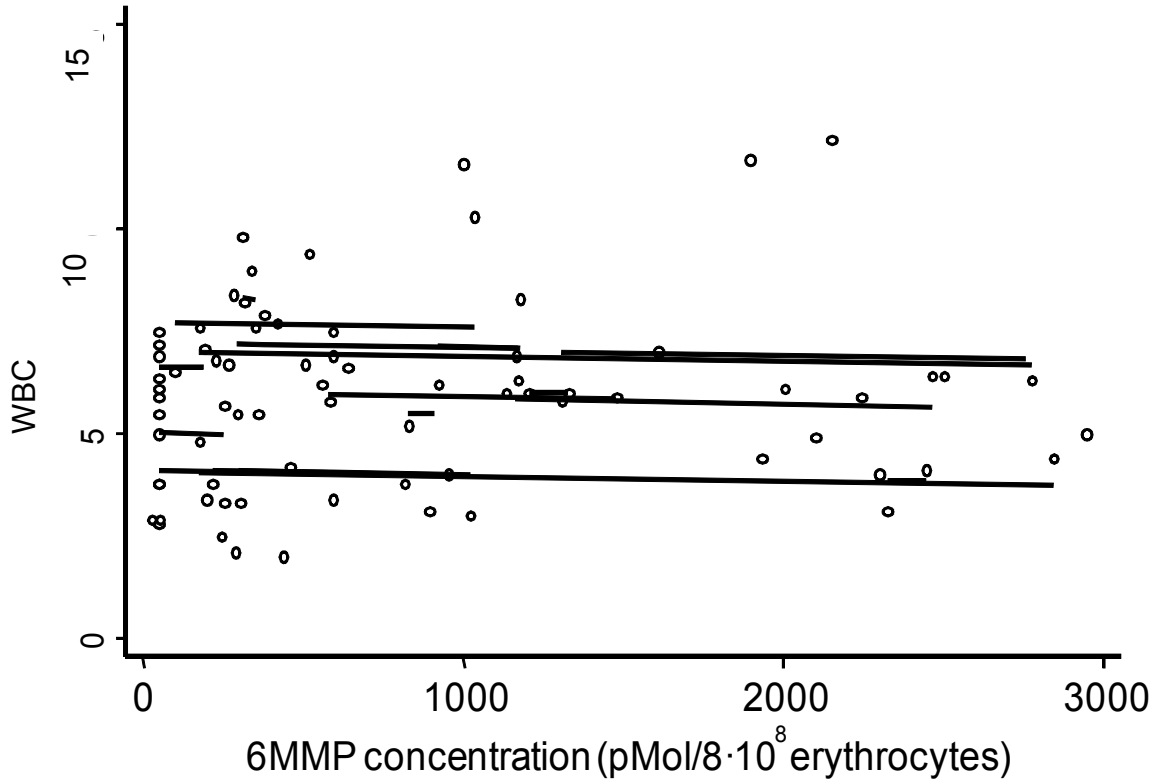


Figure 7: Association between 6-TGN concentration and current MCV, and between 6-MMP concentration and current WBC value (individual concentration-response lines are drawn only for patients with several measurements).

Finally, MCV revealed a moderate correlation with ferritin levels (determined on 490 occasions) but no association with either vitamin B12 (334 determinations) or folic acid (287 determinations), and appeared to be negatively correlated with C-reactive protein levels (1078 determinations). On the other hand, WBC counts were positively correlated with C-reactive protein levels.

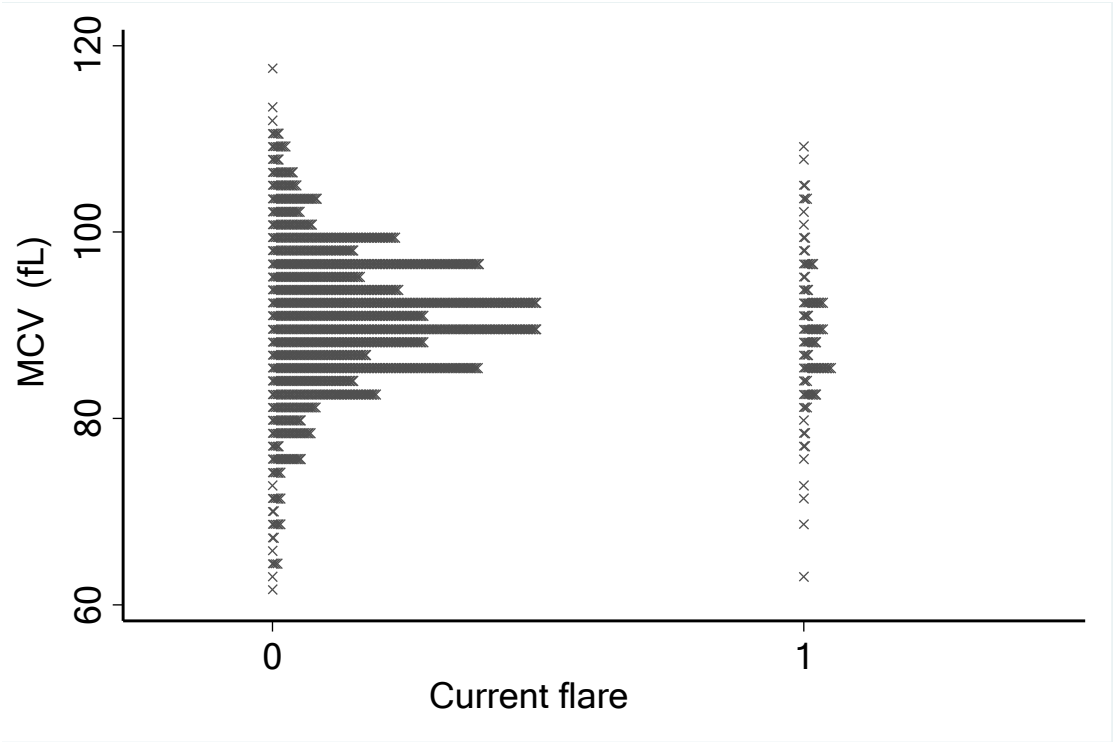
Relationship between MCV, WBC and flares

Current MCV or WBC and flare occurrence

Our hierarchical logistic regression analysis found a significant association between a low current MVC value and the probability of flare occurrence, with an odds ratio of 0.964

corresponding to a relative risk of 3.7% for every fL of decrease in MCV (p=0.017). Similarly, a higher current WBC level was associated with a higher probability of flare with an odds ratio of 1.08, translating into a 8% increase for every supplemental G/L of WBC (p=0.009).

We observed no further relevant associations combining MCV or WBC with time, AZA or 6MP doses, their history or their change during past months (analyses not detailed here).



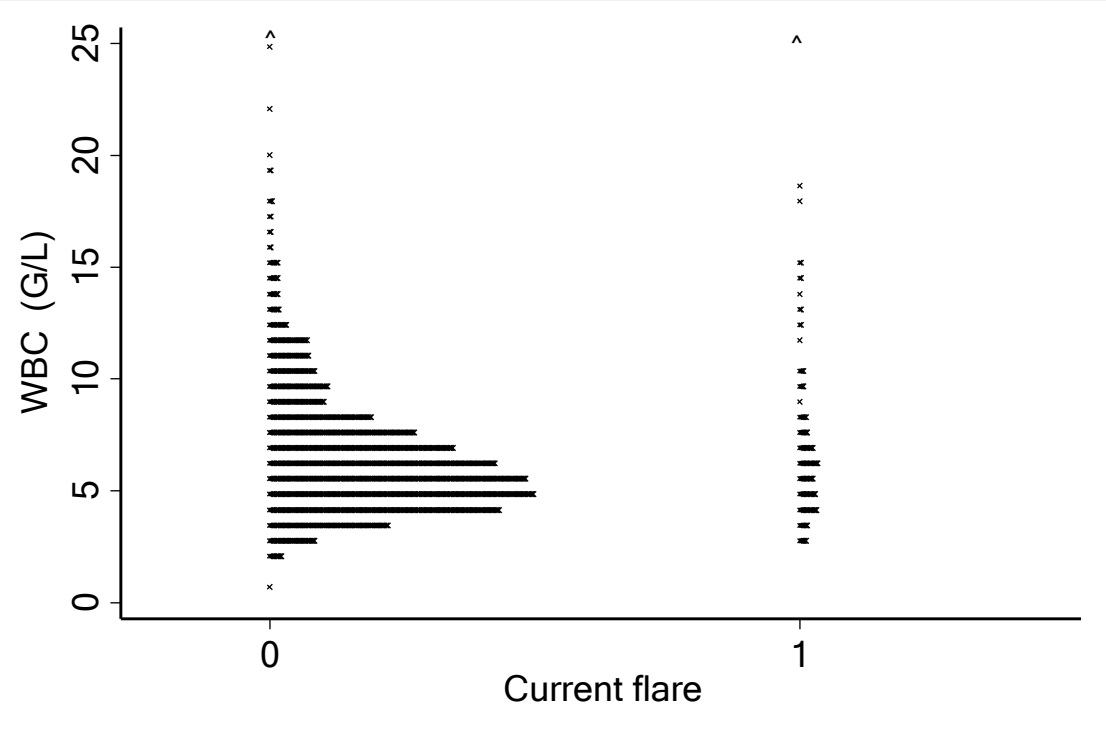
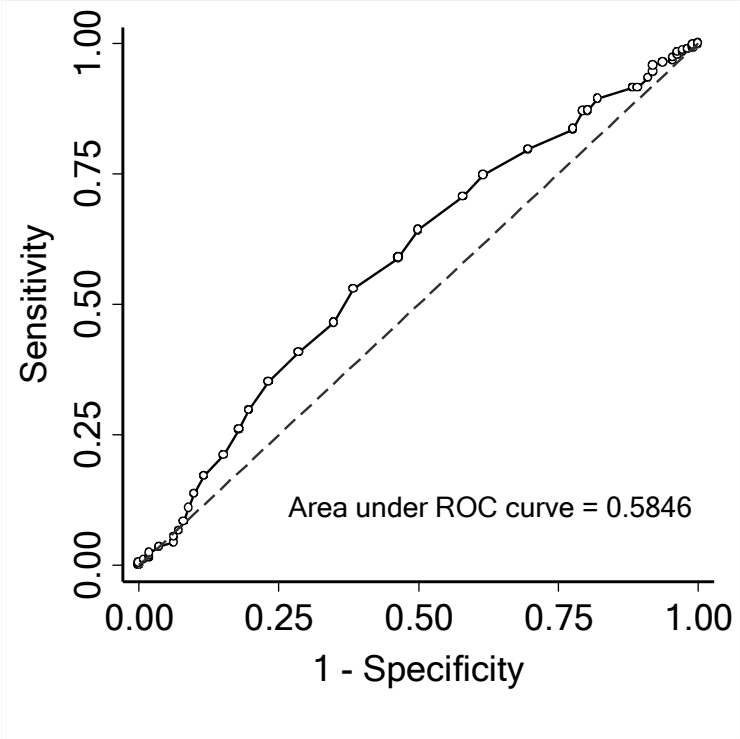


Figure 8: Current MCV or WBC and contemporaneous flare occurrence (0 = no flare, 1 = flare on measurement time)

The MCV or WBC effect on the prediction of current flare can also be represented with receiver operating characteristic (ROC) curves, showing the rather limited predictive power of MCV, and the absence of real predictive power of WBC regarding the occurrence of flares.



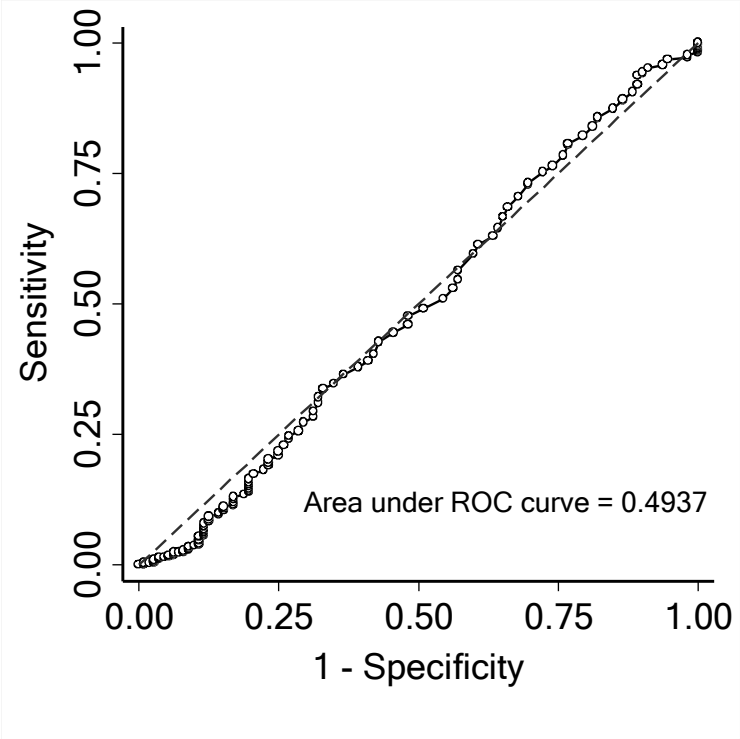


Figure 9: ROC curves for current MCV and WBC levels in flare prediction

History of MCV or WBC and flares occurrence

The MCV effect appeared to be maximal for current MCV and decreased progressively for MCV values integrated over longer duration, losing statistical significance after one month. Conversely, changes in mean MCV between the current and the previous months improved the prediction independently from current MCV (p=0.04). Changes over 2x2 months or 2x3 months were non-informative, though.

Looking for a similar memory effect of WBC prediction of flares, we observed an association increasing up to the mean of 3 months (p=0.025). Change in mean WBC over 2x2 months or 2x3 months improved prediction independently from the current WBC. On the contrary, WBC change between the current and the previous months was non informative.

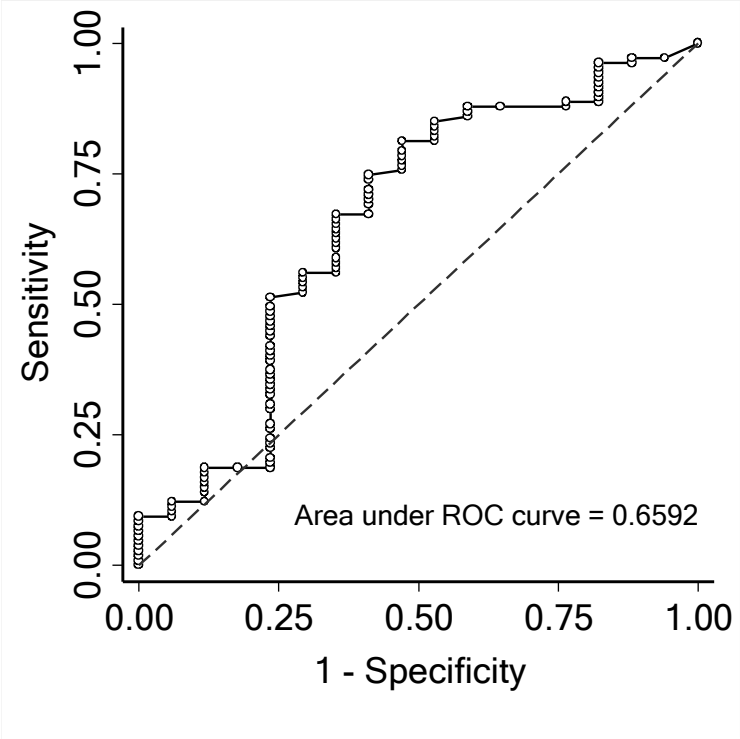
Applying a similar approach to changes of MCV compared to pre-treatment value, when available, failed to identify this difference as a flare predictor (p=0.44). Conversely, for patients having provided a value of WBC before treatment, we could observe that WBC difference versus baseline under thiopurine treatment was a flare predictor (p=0.017).

Current 6 thioguanine and 6 mercaptopurine concentration and occurrence of flare

This analysis showed an association, however of limit significance (p=0.05) between low levels of the 6TGN metabolite concentrations and the probability of flare occurrence, on pooling AZA and 6MP treatments. However, this evaluation was based on only 124 measures, thus limiting its statistical power. Some association persisted over up to 6 months on relating the current occurrence of flare with past concentration determinations. The levels of the 6MMP metabolite revealed no correlation with flare occurrence (p=0.2).

We observed no further relevant trend combining 6TGN, 6MMP concentration with time, AZA or 6MP doses, their history or their change during past months (analyses not detailed here).

The 6TGN and 6MMP based predictions of a current flare can also be represented with ROC curves. This analysis reveals a clearly better performance for 6TGN concentrations than for MCV or WBC, while the levels of 6MMP are not predictive of flare occurrence.



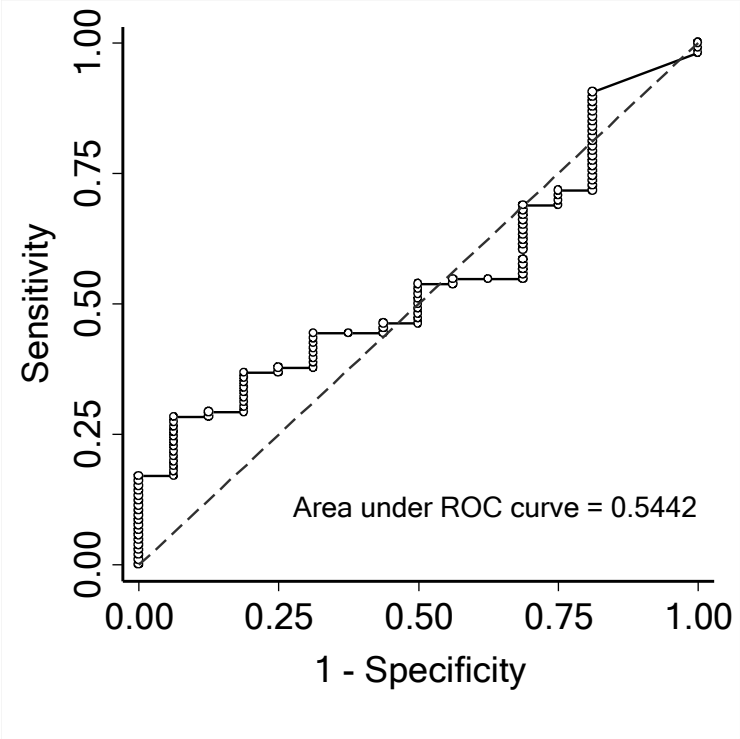


Figure 10: ROC curves for current 6TG and 6MMP concentrations for flare prediction

The occurrence of flare was not associated with the past history of 6TGN, 6MMP concentrations.

Finally, “flares “also tended to occur more frequently in association with high C-reactive protein (p=0.001, 1006 determinations analyzed by mixed-effect linear regression accounting for patient effect) or sedimentation rate level (p=0.003, 267 determinations), and with low hematocrit or haemoglobin concentration (p=0.007) (results not detailed). None of these covariables improved the prediction of flare occurrence by MCV or WBC values.

Discussion

MCV is a measure of the average red blood cell size and is calculated by dividing the haematocrit by the red blood cell count. As shown in previous studies, an increase in MCV is commonly observed during thiopurine therapy (11; 16). This study aimed to assess whether this change in MCV could be used as a monitoring tool during thiopurine therapy. In parallel, we analyzed the performances of WBC, a marker usually followed up to detect toxicity, regarding treatment efficacy.

Macrocytosis is a frequent side effect seen in therapies such as azathioprine, sulfalazine, other sulfa-based medications and methotrexate even in patients with normal vitamin B12 and folate concentration, and it is accompanied with megaloblastoid changes in bone marrow (21; 29), primarily in erythroid precursors (35). The mechanism for this increase in MCV is presumably mediated by interference with DNA synthesis and resulting inhibition of cell proliferation and maturation (34). Clinicians have anecdotally noted macrocytosis in patients treated with thiopurine, but little has been reported in the medical literature. It may take at least three months of thiopurine therapy before an increase in MCV is observed (11; 30), in line with the normal erythrocyte life span of 120 days.

We confirm that MCV consistently and dose-dependently increases under thiopurines treatment by an average of about 6 fL. This effect tends to become more pronounced over treatment duration, with patients following variable individual trajectories along the time. This biomarker could thus be observed to confirm and monitor therapeutic exposure. However, the predictive performance of MCV regarding the occurrence of IBD event remains fairly limited, leaving this marker with a questionable role for clinical optimization of thiopurines treatment. Expressing ongoing-treatment MCV values as differences versus pre-treatment baseline does not improve their predictive value either. Interestingly, while the effect on MCV seemed related with the history of thiopurine exposure over several months, the occurrence of IBD flares appeared exclusively correlated with current MCV level, without association with previous values. The inertia characterising MCV changes seems thus to have no counterpart regarding treatment efficacy. While low iron stores, reflected in ferritin levels, tended to counteract the effect of thiopurines on MCV by promoting microcytosis, neither vitamin B12 nor folic acid appeared to play any

confounding role regarding MCV levels.

Due to reversible myelosupression (27) monitoring for cytopenias, especially leukopenia, is essential in patients taking thiopurine. Physicians recommend weekly complete blood counts for the initial month of drug therapy with monthly blood count checks thereafter (28).

More frequent remission and more rapid achievement of remission has been described after the development of leukopenia, while the practical significance of leukopenia or neutropenia for treatment monitoring remains rather controversial (4; 24; 25; 31; 32; 33).

Our investigation found a dose dependent, significant leukopenia by an average of 2.4 G/L under treatment. Despite an interesting association between flare occurrence and the current level and history of WBC count, we found a negligible predictive performance of WBC regarding the occurrence of flare, as illustrated by our ROC analysis. WBC levels tended to decrease over treatment duration but, unlike MCV, they revealed no individual variability among patient trajectories. This observation is in line with the much shorter kinetics of WBC turnover, and means that delaying WBC check is of no interest to capture any progressive, mid- or long-term drift.

Measurement of thiopurine metabolite concentration for optimization of drug therapy was not infrequently requested in our study patients, although its interest for therapeutic monitoring remains controversial (22). Some authors found no predictive value of thiopurine metabolites for clinical response (23; 26). Like other studies that suggest a correlation between 6-TGN concentrations and clinical response (24; 25), our investigation found an association with the occurrence of flare, which appeared stronger than for MCV, although still moderate. Noticeably, 6-TGN concentration also correlated with MCV, better than did AZA dosage. This points clearly towards the importance of pharmacokinetic variability to explain variations in both treatment response and MCV changes. Moreover, the association between 6-TGN concentration and flare occurrence remained stable over up to 6 months of time lag. This tends to confirm the potential interest of 6-TGN measurement for the therapeutic monitoring of thiopurine therapy. While this pharmacokinetic marker appears superior to MCV, it is however more costly and technically demanding, thus raising cost/utility issues. Circulating concentrations of 6MMP

were associated neither with flare occurrence, nor with MCV levels, but they slightly correlated with WBC decrease.

The main limitations of our study are the limited number of patients studied (140), its retrospective design, the absence of structured sampling schedule and of a central laboratory, and the necessity of manual recording especially for MCV data. It is possible that inflammatory status or iron stores of the patients confounded to some extent the MCV values observed. Vitamin B12 or folate deficiency might also have affected MCV without relation with efficacy or dosage of thiopurine drugs, although we found no indication for this. Chronic alcohol consumption could also directly affect MCV (38), but no corresponding information was available regarding our study patients to take into account this potential confounding factor.

The absence of a uniform definition of IBD flare in the literature, due to the proteiform clinical manifestations of this disease and the absence of specific diagnostic tests, also represented a difficulty, that obliged us to create a composite outcome using available clinical criteria.

Another potential bias reported in studies using corticosteroid sparing or withdrawal (31; 32) as a criterion to define remission is that the fall of leucocytes could be explained by the decrease of steroid dose, independently of thiopurine treatment. The fact that our patients where under thiopurine treatment from at least 3 month would however diminish the risk of a such bias.

In conclusion, MCV and WBC are markers that deserve attention to check and monitor therapeutic exposure to thiopurine drugs in IBD patients. However, their predictive performance regarding the occurrence of IBD events seems too limited to recommend them as a privileged monitoring tool for treatment optimization. Further prospective studies evaluating the systematic measurement of metabolite concentration might better contribute to further improve efficacy and refine dosage adjustment during thiopurine therapy.

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