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LETTER TO THE EDITOR

Fluorescein angiography leads to increased fluorescence of blood cells and may hamper routine haematology analysis of ophthalmology patients

Dear Editors,

Haematology analysers are routinely applied in healthcare, using the complete blood count (CBC) as a common screening parameter. Haematology analysers adopt a variety of techniques to classify blood cells, including optical light scattering and blood cell fluorescence (with and without added reagents).[1] Interference in the measurement can lead to abnormal CBC results and thus to incorrect diagnoses and unnecessary treatments. In this study, we describe an unexpected CBC interference that occurs after a routine ophthalmic examination. Fluorescein angiography (FA) and indocyanine green angiography (ICGA) are imaging modalities frequently used for the diagnosis and monitoring of several eye diseases involving the retina and choroid.[2] Sodium fluorescein is a small molecule that extravasates in the choroid and, in case of damaged retinal vessels or neovascular lesions, leaks out of these vessels. FA is mainly suitable to visualize (sub)retinal structures, since the blue excitation light and the green fluorescence are partially absorbed by the retinal pigment epithelium. The near-infrared wavelength for excitation and emission of indocyanine green (ICG) molecules is relatively long. Therefore, is it possible to penetrate the retinal pigment epithelium and visualize the underlying choroidal structures.[3] Central multifocal choroiditis (cMFC) is a rare form of non-infectious uveitis predominantly affecting young myopic women. In this disease, FA and ICGA are frequently performed to monitor the choroidal inflammation and the complication of choroidal neovascularization.[2] Patients are often treated with immunomodulatory agents, requiring regular blood analysis for the monitoring of side effects. Question is to what extent the outcome of routine CBC analysis in ophthalmology patients is affected by FA and ICGA.

In this study, we included patients diagnosed with cMFC who were incorporated in the Utrecht Patient Oriented Database (UPOD) in the UMC Utrecht, Utrecht, the Netherlands. The study followed the tenets of the Declaration of Helsinki and all subjects provided a written informed consent. UPOD collects and stores the results from the Abbott Cell-Dyn Sapphire automated blood cell analysers. The blood cell analyser is equipped with an integrated 488-nm blue diode laser and uses spectrophotometry, electrical impedance, laser light scattering (multi angle polarized scatter separation) and 3-colour fluorescent technologies to measure morphological parameters of leukocytes, red blood cells and platelets for classification and enumeration.[4] Data were collected from the electronic patient record system concerning demographic information and information about FA and ICGA including the time between the intravenous administration of fluorescein and ICG dye and CBC analysis by the haematology analyser. Standard dye dose consisted of 4 mL of 100 mg/mL sodium fluorescein and either 4 mL of 5 mg/mL indocyanine green or 6.5 mL of 2.5 mg/mL infracyanine green. The patients were stratified in three groups: a group without FA and ICGA within 8 hours prior to the analysis of CBC (FA−/ICGA−), a group with exclusively ICGA prior to CBC analysis (FA−/ICGA+) and a group with both FA and ICGA prior to CBC analysis (FA+/ICGA+). The raw CBC data from UPOD, including research only parameters, were used as an outcome. Data analyses were performed in RStudio version 1.2.5001 (RStudio Team) and R version 3.6.1 (R Foundation for Statistical Computing). A likelihood ratio test (LRT) with a false discovery rate of 5% was applied for all the raw CBC parameters to perform differential abundance analysis for the 3 groups including the covariates age and sex. Linear regression analysis was performed in FA+/ICGA+ and FA−/ICGA+ patients to evaluate the gradient of the associated CBC parameters over time. All fluorescence-related CBC parameters were measured in arbitrary units (AU).

A total of 122 patients with cMFC were included in this study with a median (range) age of 42 (16-80) years and 104/122 (85%) were female. The FA−/ICGA− group consisted of 82 patients, FA−/ICGA+ of 4 patients and finally the FA+/ICGA+ comprised 36 patients. Differential abundant analysis revealed several fluorescence-related parameters that were significantly different between the three groups: mean neutrophil fluorescence (NFMN), mean lymphocyte fluorescence (LFMN), mean monocyte fluorescence (MFMN) and mean erythrocyte fluorescence (RBCFMN) (Table 1). Note that the time between the administration of fluorescein and/or indocyanine green dye and CBC analysis was not significantly different between the FA+/ICGA+ and FA−/ICGA+ groups ($P = .56$) (Table 1).

All fluorescence-related parameters were exclusively increased in patients in the FA+/ICGA+ group. The difference for

TABLE 1 Fluorescence-related parameters that were increased after the performance of FA and ICGA

	FA+/ICGA+		FA−/ICGA+		FA−/ICGA−		
	Median	(IQR)	Median	(IQR)	Median	(IQR)	*P*-value[a]
NFMN	83.0	(17.3)	68.7	(1.7)	69.3	(2.5)	3.13×10^{-43}
LFMN	58.9	(23.6)	37.9	(6.1)	38.5	(8.3)	5.75×10^{-34}
MFMN	77.3	(24.1)	54.1	(7.6)	52.9	(6.6)	2.77×10^{-49}
RBCFMN	86.8	(5.4)	85.3	(3.1)	84.9	(4.5)	2.25×10^{-2}
Time (hours)[b]	2.8	(1.3)	3.3	(2.1)	NA	NA	0.56[c]

Note: Fluorescence-related parameters that were significantly different between patients undergoing FA and ICGA (FA+/ICGA+) within 8 hr prior to CBC analysis, patients undergoing exclusively ICGA (FA−/ICGA+) and patients who did not undergo FA or ICGA prior to CBC analysis (FA-/ICGA-). All fluorescence-related parameters are quantified in arbitrary units. The time between the administration of fluorescein and/or indocyanin green dye was not significantly different for the FA-/ICGA+ and FA+/ICGA+ group.

Abbreviations: CBC, complete blood count; FA, fluorescein angiography, ICGA, indocyanine green angiography; IQR, interquartile range; LFMN, mean lymphocyte fluorescence; MFMN, mean monocyte fluorescence; NFMN, mean neutrophil fluorescence; RBCFMN, mean erythrocyte fluorescence.

[a]Results of the likelihood-ratio test after false discovery rate correction of 5% and with the covariates age and gender incorporated in the model.

[b]The time between the injection of indocyanine green dye (and if applicable fluorescein dye) and the CBC analysis by the haematology analyser.

[c]The results of the t-test for the FA+/ICGA+ and FA−/ICGA+ group.

NFMN between the 3 groups is visualized in Figure 1A. In patients who underwent ICGA (either with or without FA), linear regression analysis was performed for the time between the intravenous injection of ICG and (if applicable) fluorescein dye and CBC analysis and NFMN. This analysis revealed a significant negative association (beta −0.11 AU/minute (95% CI −0.15 to −0.07), intercept=107.0, $P = 3.9 \times 10^{-6}$, Adj. $R^2 = 0.42$). The decline of NFMN over time is exclusively present in the FA+/ICGA+ group and was not observed in the FA−/ICGA+ group (Figure 1B). Similar results were found for the monocytes, lymphocytes and erythrocytes (data not shown).

In this study, we found that after intravenous injection of fluorescein dye for ocular angiography, patients demonstrated altered fluorescence-related parameters explored with a routine haematology analyser. The most predominant alterations were an elevated mean fluorescence of neutrophils, monocytes and lymphocytes. On the contrary, in patients who received solely ICG dye, these alterations were not observed. This is probably due to the fact that the excitation and emission peaks of ICG are 789 nm and 814 nm, respectively, which are out of range of the Cell-Dyn Sapphire fluorescence detectors. The fluorescence of the blood cells is measured using a fluorescence detector (FL3 channel) with a wavelength of 615-648 nm and the excitation and emission peaks of fluorescein are 494 nm and 521 nm, respectively. We suggest that particularly the monocytes, neutrophils and lymphocytes take up the fluorescein dye following fluorescein angiography. The fluorescein molecules are strongly polar and diffuse only slowly through the cell membrane.[5] Therefore, the mechanism of entry probably involves facilitated diffusion or active transport across the cell membrane.[6] Previously, Meisingset and Steen[5] estimated that the fraction of intracellular fluorescein bound to proteins or associated with cell structures was 70%, the rest of the fluorescein being dissolved in the cellular water. Subsequently, caused by the strong fluorescence intensity of fluorescein, fluorescein in these cells is measured in the FL3 channel as an increased mean fluorescence of these cells. Our study demonstrates that this alteration in fluorescence measurement in these cells is present at least up until 6 hours after fluorescein angiography. This is the first study in a large group of patients that confirms the disturbance of fluorescence-related parameters with a routine haematology analyser. Similar results were described in a case report.[7] In this case report, the increased fluorescence in the lymphocytes, monocytes, and granulocytes was seen as long as 20 hours after fluorescein injection. The interference of fluorescent dyes has also been reported in the context of analytical flow cytometry impairing a proper discrimination of lymphocyte subsets labelled with fluorochrome-conjugated antibodies.[6,8] Fluorescence-related measurements are only performed in the context of medical research, and consequently, we think FA will not have great impact on the relevant clinical laboratory results. However, current generation haematology analysers used in routine analysis of the CBC use fluorescent techniques for the classification of bloods cells. Moreover, it cannot be ruled out that fluorescein dye influences other biochemical processes and alters additional laboratory results. Thus, if possible, we would advise to perform CBC analysis prior to the intravenous injection of fluorescein dye. Moreover, if CBC analysis demonstrates unexpected results, ophthalmologists should always consider interference with fluorescein dye as a possible explanatory factor and could repeat the CBC analysis on a day fluorescein dye is not administered to the patient. In the future, it would be interesting to compare CBC

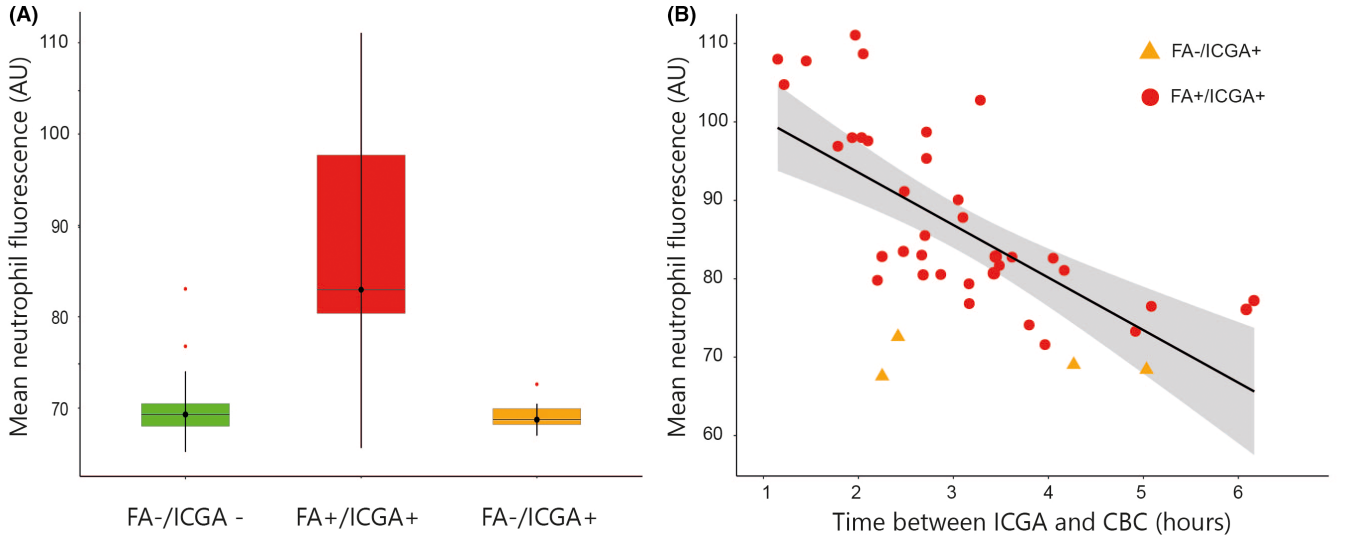

FIGURE 1 (A) The difference in NFMN between patients undergoing FA and ICGA (FA+/ICGA+) within 8 hr prior to CBC analysis, patients who underwent exclusively ICGA (FA−/ICGA+) and patients who did not undergo FA or ICGA prior to CBC analysis (FA−/ICGA−) including the group median, interquartile range and outliers (red dots). (B) The relationship between NFMN and the time between ICGA (and if applicable FA) and CBC analysis. The black line represents the linear model including the 95% confidence interval (grey area). The red points represent patients in the FA+/ICGA+ group (n = 36), and the orange triangles represent patients in the FA-ICGA+ group (n = 4). Abbreviations: AU, arbitrary units; FA, fluorescein angiography; ICGA, indocyanine green angiography; CBC, complete blood count; NFMN, mean neutrophil fluorescence

data prior to and shortly after the administration of fluorescein dye within the same patient to explore which parameters are most severely influenced by fluorescein dye in a prospective design.

KEYWORDS

Abbott Cell-Dyn Sapphire, CBC, complete blood count, flow cytometry, fluorescein angiography, fluorescence

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CONFLICT OF INTEREST

Author Saskia Haitjema received a fellowship by Abbott Diagnostics and the Central Diagnostic Laboratory has contracts with Abbott Diagnostics.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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