

## Influence of a light meal on routine haematological tests

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**Introduction.** Patient-related variables, such as physical exercise, stress and fasting status are important sources of variability in laboratory testing. However, no clear indications about fasting requirements exist for routine haematological tests, nor has the influence of meals been assessed.

**Methods.** We studied 17 healthy volunteers who consumed a light meal containing a standardized amount of carbohydrates, protein and lipids. Blood was taken for routine haematological tests before the meal and 1, 2 and 4 hours thereafter.

**Results.** One hour after the meal, neutrophil count and mean corpuscular haemoglobin (MCH) increased significantly, whereas lymphocyte and monocyte counts, red blood cell distribution width, haematocrit, and mean corpuscular volume decreased significantly. A clinically significant variation was only observed for lymphocytes. Two hours after the meal, a significant increase was observed for neutrophils and MCH, whereas lymphocytes, eosinophils, haemoglobin and haematocrit decreased significantly. Clinically significant variations were recorded for lymphocytes, red blood cells (RBC), haemoglobin, haematocrit and MCH. Four hours after the meal MCH was significantly increased, while lymphocytes, eosinophils, RBC, haemoglobin and haematocrit were significantly decreased. Clinically significant variations were recorded for neutrophils, eosinophils, RBC, hematocrit and MCH.

**Conclusion.** The significant variation of several haematological parameters after a light meal demonstrates that the fasting time needs to be carefully considered in order to interpret the results of haematological tests correctly.

**Key Words:** fasting, haematological tests, meal, pre-analytical variability.

### Introduction

Healthcare is an extremely complex process, involving a kaleidoscope of medical disciplines. Traditionally, medical errors are identified as incorrect diagnoses, mishandled clinical procedures or, globally, as results of inappropriate clinical decision-making. Laboratory diagnostics, like other medical areas, is often delivered in a high-pressure, fast-moving environment, involving a vast array of innovative and complex technologies, so that it is no safer than other

areas of healthcare<sup>1-2</sup>. Under some circumstances things can go wrong within the total testing process, producing spurious results and, potentially, unintentional harm to the patients<sup>3-5</sup>. Although errors may occur in each phase of laboratory diagnostics, there are several lines of evidence suggesting that the vast majority of errors arise during the manually intensive pre-analytical phase, mostly because of poor standardisation, mishandled procedures and lack of adherence to best practice guidelines<sup>6-8</sup>.

An accurate investigation of haematological disorders requires appropriate and discretionary use of laboratory resources. However, total quality in haematological testing is a prerequisite for clinically reliable results<sup>9</sup>. Modern automated haematology counters provide clinicians quickly with results that are characterized by a high degree of precision and accuracy<sup>10</sup>. Spurious results may occasionally be observed in some circumstances, such as agglutination in the presence of ethylenediamine tetra-acetic acid (EDTA), cryoglobulins, lipids, insufficiently lysed red blood cells (RBC), erythroblasts and platelet aggregates<sup>11,12</sup>. Pre-analytical variability is another important source of errors in haematological testing, accounting for 0.36%<sup>7</sup> to 0.45%<sup>9</sup> of all unsuitable specimens referred for routine haematological testing. Current data on pre-analytical errors in routine haematological testing indicate that undue clotting, samples collected into unsuitable containers or at an inappropriate volume (either insufficient volume for testing or suboptimal blood to anticoagulant ratio) are the prevailing causes of unsuitable specimens<sup>7</sup>. Patient-related variables, such as physical exercise, stress and fasting status are additional sources of variability in laboratory testing. The Clinical and Laboratory Standards Institute (CLSI) currently recommends a patient's fasting status, especially for those tests that are more likely to be affected by food ingestion, such as assays of glucose, lipoprotein fractions and triglycerides. For glucose alone, a fast of 4 hours is sufficient<sup>13</sup>. According to the National Cholesterol Education Program (NCEP), the fasting period prior to the measurement of lipids should be at least 9 to 12 hours<sup>14</sup>. Nevertheless, no clear indications about fasting requirements exist for routine haematological tests, nor has the influence of meal ingestion been previously assessed. The present study was, therefore, designed to evaluate the influence of a regular, light meal on haematological tests.

## **Material and Methods**

### **Study design**

The study population consisted of 17 healthy volunteers (8 women and 9 men; mean age  $\pm$  standard deviation: 29 $\pm$ 4), who were enrolled among the laboratory staff and gave written consent to testing. The study was also approved by our local ethical committee. Blood samples were collected by a single,

expert phlebotomist, using a 20 G straight needle (Terumo Europe NV, Leuven, Belgium), directly into 3.0 mL siliconised vacuum tubes containing 5.9 mg K<sub>2</sub> EDTA (Terumo Europe NV, Leuven, Belgium). A first blood sample was collected between 8:00 and 8:30 a.m. after an overnight fast. Immediately after blood collection, the volunteers consumed a light meal, containing standardised amounts of carbohydrates, protein and lipids (Table I). The meal was based on commercial food regularly purchased at a shop, and included one slice of cheese, one yogurt, two slices of bread, a chocolate snack and a fruit juice. The precise composition of the meal is shown in table I. Subsequent blood samples were collected 1, 2 and 4 hours after the end of the meal. Each phase of sample collection was carefully standardised, including the use of needles and vacuum tubes from the same lot. No specimen needed to be discarded due to unsatisfactory attempts, difficulty in locating a suitable venous access or missing the vein.

### **Laboratory testing**

All samples were processed for routine haematological testing immediately after collection (<15 min) on the same Advia 2120 automated haematology analyser (Bayer Diagnostics, Newbury, Berkshire, UK). The parameters tested included haemoglobin, haematocrit, RBC count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), platelet count, mean platelet volume, RBC distribution width (RDW), white blood cell (WBC) count and WBC differential, including neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells. The instrument was calibrated against appropriate proprietary reference standard material and verified with the use of proprietary controls. A multicenter evaluation of the within-run precision of the Advia 2120 system showed coefficients of variation ranging from 1.6% to 2.3% for WBC, from 2.1% to 2.8% for platelets, from 0.6% to 0.9% for RBC and always lower than 0.7% for hemoglobin, MCV and MCH<sup>15</sup>. Results are shown as mean and standard error of the mean (SEM).

### **Statistical analysis**

The significance of differences between samples was assessed by paired Student's t-test. The level of statistical significance was set at  $P < 0.05$ . Finally,

**Table I** - Nutritional composition of the light meal

Nutritional composition	Slice of cheese	Yogurt	Slice of bread	Chocolate snack	Fruit juice	Total
Number (overall weight)	1 (25 g)	1 (125 g)	2 (46.8 g)	1 (20.7 g)	1 (200 g)	417.5 g
Kcal	63.75	134	126	105	134	562.75
KJ	266,25	562	532	438	572	2370
Protein (g)	4.4	4.1	4,2	1.1	0,8	14.6
Carbohydrate (g)	0.8	19.4	22	12.7	32	86.9
Sugar (g)	0.8	12	3	10	10	35.8
Total lipids (g)	4.6	4.4	2.4	5.5	0	16.9
Saturated lipids (g)	3.125	N/A	0.8	3.7	0	-
Fibre (g)	0	N/A	0.9	0.2	2	-
Sodium (g)	0.3	N/A	0.286	0.02	0	-
Calcium (g)	0.133	0.131	N/A	N/A	N/A	-

the biases 1, 2 and 4 hours after intake of a standardised meal were compared with the current desirable quality specifications for bias, derived from biological variation<sup>16</sup>.

## Results

The results of this investigation are shown in table II. One hour after the ingestion of the meal, significant increases were observed in neutrophils and MCH, whereas lymphocytes, monocytes, RDW, haematocrit and MCV were significantly decreased (Figures 1 and 2). However, a clinically significant variation, as compared with the current desirable quality specifications<sup>16</sup>, was only observed for lymphocytes. Two hours after the ingestion of the meal, the neutrophil count and MCH remained significantly increased, whereas lymphocyte and eosinophil counts, haemoglobin and haematocrit were significantly decreased (Figures 1 and 2). Clinically significant variations were recorded for lymphocyte and RBC counts (although the decreases were not statistically significant according to the Student's paired t-test), haemoglobin, haematocrit and MCH. Four hours after the ingestion of the meal a significant increase was recorded only for MCH, while lymphocytes, eosinophils, RBC count, haemoglobin, haematocrit mean platelet volume were significantly decreased. Clinically significant variations were recorded for

neutrophils (although this increase was not statistically significant according to Student's paired t-test), eosinophils, red blood cell count, haematocrit, MCH and mean platelet volume.

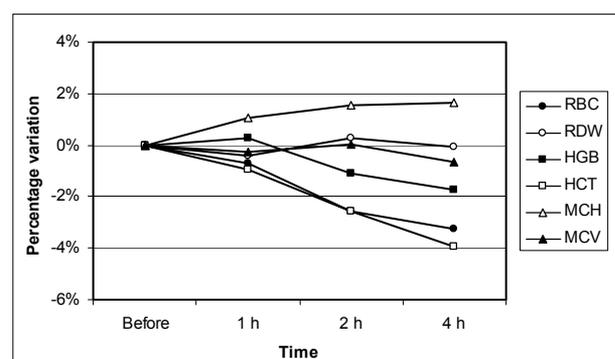
## Discussion

Laboratory testing is the mainstay for the screening, diagnosis and follow-up of numerous haematological disorders. A high degree of quality throughout the entire testing process is, therefore, crucial in order to provide clinicians with reliable laboratory data<sup>10</sup>. As in other areas of laboratory diagnostics, however, pre-analytical variability can affect haematological tests, thereby producing spurious results that can jeopardise patients' safety<sup>1,2,5,8</sup>. Although an adequate period of fasting before collecting blood is advisable, especially when performing tests that might be influenced by ingestion of carbohydrates and lipids (e.g., assays of glucose and triglycerides)<sup>13,14</sup>, no information is available on post-prandial variations of routine haematological parameters. This might be relatively irrelevant in most circumstances, when blood is collected in the morning from fasting patients. However, there might be some cases when blood must be collected from non-fasting patients, such as those suffering from urgent clinical conditions that require stat testing. In such cases, familiarity with post-prandial variations of the

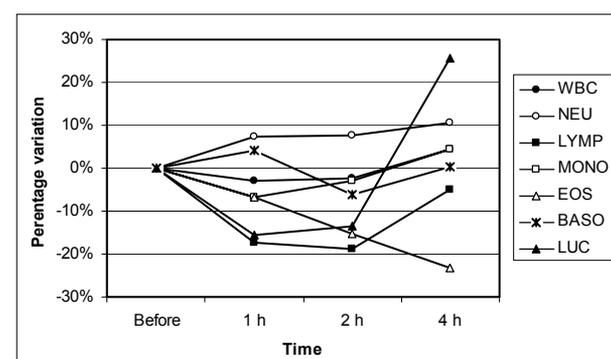
**Table II** - Post-prandial variation of the routine haematological profile after a light meal

	WBC	NEU	LYMP	MONO	EOS	BASO	RBC	RDW	HGB	HCT	MCV	MCH	PLT	MPV	
Desirable Bias (%)	5.6	9.0	7.4	13.2	19.8	15.4	1.7	1.7	1.8	1.7	1.2	1.4	5.9	2.3	
<b>Baseline specimen</b>															
Mean value (SEM)	6.97 (0.63)	3.89 (0.43)	2.24 (0.18)	0.36 (0.04)	0.13 (0.02)	0.04 (0.00)	4.72 (0.10)	13.11 (0.27)	13.64 (0.29)	39.66 (0.71)	83.96 (1.39)	28.87 (0.60)	271.20 (16.46)	8.27 (0.12)	
<b>1 h after meal</b>															
Mean value (SEM)	6.76 (0.56)	4.18 (0.42)	1.85 (0.15)	0.33 (0.03)	0.12 (0.02)	0.04 (0.00)	4.69 (0.09)	13.06 (0.26)	13.68 (0.29)	39.27 (0.70)	83.73 (1.37)	29.18 (0.62)	269.70 (15.36)	8.35 (0.10)	
Mean % difference	-3	7.4	<b>-17.4</b>	-6.9	-6.8	4.2	-0.7	-0.4	0.3	-1	-0.3	1.1	-0.6	1	
<i>p</i>	0.129	<b>0.009</b>	<b>0.000</b>	<b>0.014</b>	0.085	0.413	0.066	<b>0.007</b>	0.247	<b>0.035</b>	<b>0.011</b>	<b>0.000</b>	0.284	0.112	
<b>2 h after meal</b>															
Mean value (SEM)	6.80 (0.53)	4.19 (0.41)	1.82 (0.16)	0.35 (0.03)	0.11 (0.02)	0.04 (0.00)	4.60 (0.09)	13.15 (0.26)	13.49 (0.28)	38.62 (0.70)	83.98 (1.37)	29.32 (0.59)	277.58 (15.78)	8.23 (0.14)	
Mean % difference	-2.4	7.6	<b>-18.7</b>	-3.0	-15.4	-6.2	<b>-2.6</b>	0.3	-1.1	<b>-2.6</b>	0.0	<b>1.6</b>	2.4	-0.4	
<i>p</i>	0.205	<b>0.043</b>	<b>0.000</b>	0.129	<b>0.001</b>	0.159	0.073	0.069	<b>0.021</b>	<b>0.000</b>	0.454	<b>0.000</b>	0.113	0.387	
<b>4 h after meal</b>															
Mean value (SEM)	7.27 (0.45)	4.31 (0.33)	2.13 (0.17)	0.37 (0.03)	0.10 (0.02)	0.04 (0.00)	4.57 (0.10)	13.10 (0.27)	13.40 (0.32)	38.08 (0.76)	83.41 (1.34)	29.35 (0.63)	273.86 (15.56)	8.07 (0.12)	
Mean % difference	4.3	<b>10.7</b>	-4.9	4.4	<b>-23.2</b>	0.3	<b>-3.3</b>	-0.1	-1.7	<b>-3.9</b>	-0.6	<b>1.6</b>	1.0	<b>-2.3</b>	
<i>p</i>	0.326	0.114	0.097	0.500	<b>0.003</b>	0.403	<b>0.001</b>	0.304	<b>0.021</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.325	<b>0.015</b>	

HGB: haemoglobin; HCT: haematocrit; RBC: red blood cell count; MCH: mean corpuscular haemoglobin, MCV: mean corpuscular volume; PLT: platelet count; MPV: mean platelet volume; RDW: RBC distribution width; WBC: white blood cell count; NEU: neutrophils; LYMP: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophils; LUC: large unstained cells



**Figure 1** - Percentage post-prandial variation of red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and RBC distribution width (RDW) after a light meal.



**Figure 2** - Percentage post-prandial variations of white blood cell (WBC) count, neutrophils (NEU), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC) after a light meal.

haematological profile is essential in order to be able to appreciate and troubleshoot "spurious" variations, and to interpret test results correctly, especially in the longitudinal monitoring of patients' data.

To the best of our knowledge, this is the first investigation assessing post-prandial variation of routine hematological parameters. The results of this study clearly demonstrate that even a light meal, such as that administered in this investigation, can induce significant variations in the routine haematological profile in healthy subjects. As far as regards the WBC count, we observed a clinically meaningful increase in neutrophils (7-10%), and significant decreases of lymphocytes (as much as -19%) and eosinophils (as much as -23%) up to 4 hours after the meal (Figure 2). We also recorded significant post-prandial decreases of RBC count, haemoglobin concentration and haematocrit, with the differences in RBC count and haematocrit values achieving clinical significance 2 and 4 hours after the meal, respectively (Figure 1).

There is not a single explanation to justify these heterogeneous changes. Variations in RBC count, haematocrit and haemoglobin concentration are most likely attributable to haemodilution following ingestion of foods and fluids<sup>17</sup>. The variations in leukocyte subpopulations are more difficult to interpret. In agreement with our findings, Hansen *et al.* previously observed increase in neutrophil and platelet counts lasting for more than 2.5 hours after the intake of a meal, whereas lymphocyte numbers decreased<sup>18</sup>. Van Oostrom *et al.* found a significant post-prandial increase in WBC in healthy subjects after an 8-hour oral fat load. However, while observing a substantial increase in neutrophils in the first 2 hours (142% higher than baseline), which did not return to baseline by the end of the test, they also recorded a substantial increase in lymphocytes (142% higher than baseline)<sup>19</sup>. Basically, food intake represents a marked intestinal exposure to antigens, requiring host defences. Besides local immune activation, this defence includes a co-ordinated systemic immune response, which may serve to support local immunity. The results of our investigation are, therefore, consistent with the hypothesis that a light meal might still be sufficient to promote emigration of lymphocytes, probably into extravascular abdominal tissues, where they may serve to support local immune defences. Such a process is accompanied by

mobilisation from bone marrow or demarginalisation of neutrophils<sup>18</sup>. On the other hand, the marked drop in eosinophils observed 2 to 4 hours after the meal is consistent with the hypothesis that meal-induced increase in cortisol promotes migration of eosinophils into the spleen, lymph nodes and thymus<sup>20</sup>.

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