academicJournals

Vol. 6(1), pp. 1-8, June 2015
DOI 10.5897/JCIIR2014.0068
Article Number: 590B1FF53470
ISSN 1996-0816

http://www.academicjournals.org/JCIIR

Journal of Clinical Immunology and Immunopathology Research

Full Length Research Paper

Blood tissue cytological status of prognosis and predictive markers in the natural history of solid cancer development

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Received 26 February, 2014; Accepted 26 May, 2015

Cancer is the major burden of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it. In many countries, cancer ranks the second most common cause of death following cardiovascular diseases. With significant improvement in treatment and prevention of cardiovascular diseases, cancer has or will soon become the number one killer in many parts of the world. The aim of this study was to optimize the means to support and diagnose early people living with solid cancers through blood exploration using flow cytometry. The techniques used included; complete blood count (CBC), flow cytometry and microscopy slides (smear). The data obtained from the microscopic analysis of blood cells searching for alterations after smear colored blade by May Grünwald Giemsa (MGG), revealed changes in size (anisocytosis) and shape (poikilocytosis) of erythrocytes with total absence of neutrophils. The main blood pathologies associated with these types of cancer obtained after CBC were: hypoglobinemia (30.76%), blood-concentration (18.75%) which marked the character of hypochromic blood tissue (true anemia), monocytosis (12.82%) and erythrocytopenia (12.82%). The decrease in the number of granules of polymorphonuclear cells and changes in the shapes of nuclei (lobularity) were observed in most patients, using the flow cytometry technique. Thus, alterations of blood tissue in solid cancers were identified and an algorithm for their exploitation has been developed to contribute to the understanding of natural history of solid cancer.

Key words: Solid cancer, epidemiology, cytological markers, flow cytometry.

INTRODUCTION

Epidemiological data on morbidity and mortality associated with cancer; revealed geographic and socio-cultural important gaps. This is due to differences in health behaviors, presentations of diseases and access to care (Dauchy and Curé, 2008). Cancer is the modification of genetic and epigenetic regulation systems

which induces mutations. These mutation cascades favour the neoplastic process, resulting in uncontrolled cell growth, uncontrolled loss of apoptosis and metastasis (Rigal et al., 2006). Approximately 10.9 million new cases and 6.7 million deaths per year is observed where more than 16 million new cancer cases and 10 million deaths are expected by 2020 (Walboomers et al., 1999). Despite this emergency, screening and diagnosis of cancer remains a major problem especially during its early stage. Current method for detecting tumors are based on the exploitation of data provided by molecules that appear in the blood tissue called tumor markers (Robby et al., 2012). Immunologically, Interleukin-6 (IL-6), a multifunctional cytokine has been found in human cervical cancer though, the mechanism remains elusive (Lin-Hung et al., 2001). As elderly people are most susceptible to cancer and population aging continues in many countries, cancer will remain a major health problem around the globe (Ma and Yu, 2006). Study of hematological and cytological deterioration was carried out in the department of oncology of Douala General Hospital. The sample consisted of 130 subjects in which, 100 were patients, who had not been under therapy, and 30 unsick people who served as controls. The main cancer found in sick people were: mammary adenocarcinoma (38%), cervix neoplasia (27%), nasopharyngeal carcinoma (11%), liver carcinoma (10%), Kaposi's sarcoma (8%) and rectum cancer (7%).

The overall goal of the current study was to explore the hematological and cytological alterations in six types of cancer in order to identify new diagnosis markers. Techniques like microscopy slide, blood count and flow cytometry allowed us to achieve the specific objectives: to highlight size changes such as anisocytosis, shape changes such as poikilocytosis of blood cells smear, and to identify quantitative changes in blood cells by blood cells counter. Changes in the number of granules (agranulocytosis) of abnormal nuclei (lobulations) in different types of cancer have also been identified.

METHODOLOGY

Study design

This study was an epidemiological descriptive study, which took off from February, 2012 to November, 2012. Samples which were collected in Douala General Hospital consisted to 4 ml of blood in ethyl-enediaminetetraacetic acid (EDTA) tubes via venous puncture at the middle vein of the elbow or at the dorsal metacarpal veins. All the samples were kept in a cool box until test analysis times. This study was conducted on 130 people aged between 35 to 70 years, that is, 100 patients and 30 controls. In total, 6 types of cancer were encountered among the sick patients.

Ethical consideration

An ethical consideration was obtained before the work. Registration was taken during the period of June, 2012 on a number N° 2012/08/485/CE/CNERSH/SP.

Blood count

This technique helped us to count cell inside blood. The blood collected in EDTA tubes was homogenized and sucked by an automatic counting machine and the result displayed on a screen.

Smear

This technique enabled us to control blood cells morphologies. After spreading on slides, cells were fixed and stained for morphological analysis by May Grünwald Giemsa. The analysis was done under a microscope (Olympus).

Flow cytometry

This method allowed us to determine the intra structure of each cell. The blood collected in EDTA tubes was homogenized and sucked by an automatic counting machine. Each cell was oriented toward a lazer which passed through, and this determined the intra-whole constitution of the cell. The result was then displayed on a screen as a diffraction diagram.

Statistical analysis

Statistical analysis was performed using two programs: Excel program helped in managing tables and graph pad 5 software helped to compute statistical analyses. Univariate analysis using logistic regression was performed to evaluate the association between types of cancers and epidemiologic parameters. p Values less than 0.05 were considered to be statistically significant.

RESULTS

Different types of cancer have been identified and

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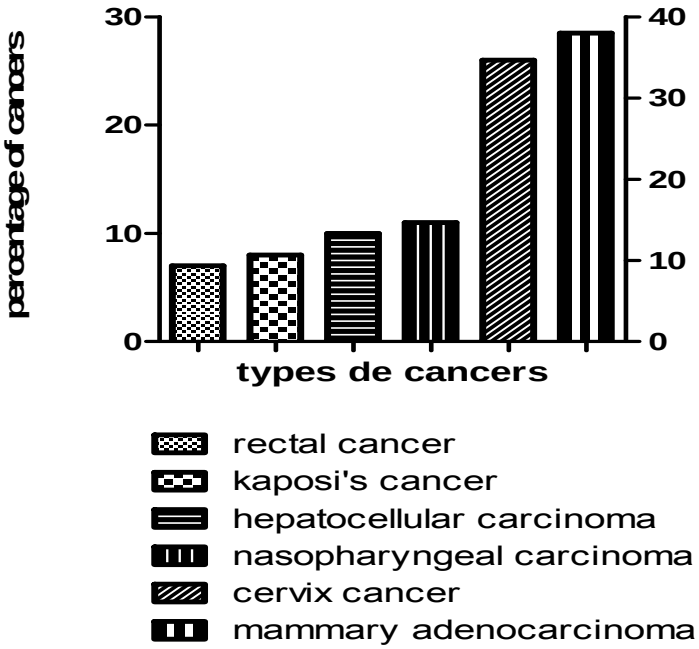

Figure 1. Different types of cancers and their percentages. Rectal cancer (7%), Kaposi's sarcoma (8%), hepatocellular carcinoma (10%), nasopharyngeal carcinoma (11%), cervical cancer (26%) and mammary adenocarcinoma (38%).

Table 1. Characterization of the sample in relation to gender and age and distribution of cancer according to age group.

Cancers types	RC	KS	CHC	NPC	NU	MAC	Total
A. Sex							
Males	2	1	0	8	0	0	11
Females	5	7	10	3	26	38	89
B. Ages							
30-40	6	8	0	0	2	12	28
40-50	0	0	1	0	14	15	30
50-60	1	0	8	5	8	10	32
60-70	0	0	1	6	2	1	10

RC: rectal cancer. KS: Kaposi's sarcoma. HCC: hepatocellular carcinoma. NPC: nasopharyngeal carcinoma. CC: cervical cancer MAC: mammary adenocarcinoma. A represents the types of cancers according to gender. The cancer of the cavum (rhinopharynx) was shown most representative with an incidence of 8 cases out of 11.Among women the cervical cancer and the adenocarcinoma were most dominating. B represent the distribution of cancers according to age group.

classified according to their percentage: Rectal cancer (7%), Kaposi's sarcoma (8%), hepatocellular carcinoma (10%), nasopharyngeal carcinoma (11%), cervical cancer (26%) and mammary adenocarcinoma (38%) (Figure 1). Types of cancer according to gender (Table 1A) showed that, the cancer of the cavum (rhinopharynx) was the most represented with an incidence of 8 cases in male out of 11. Among women, the cervical and the mammary adenocarcinoma cancer were most dominating.

The optical microscopy slide showed poikilocytosis

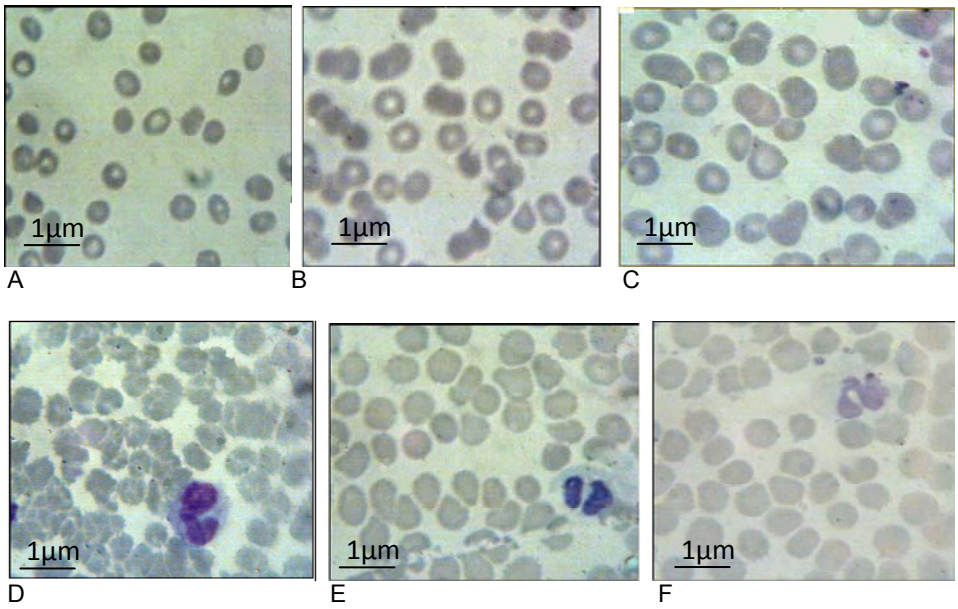

Figure 2. Results of optical microscopy slide. A: poikilocytosis microcytic, mammary adenocarcinoma, it is poikilocytosis (variation in shape) microcytic (smaller than normal cells). B: poikilocytosis dacryocytosis, cervix pathologies is observed dacryocytosis (cells tear). C: aniso-poikilocytosis micro-hypochromic, rectal cancer, the disease is observed aniso-poikilocytosis macro dacryocytoses hypochromic. D: blood concentration acanthocytosis, hepatocellular carcinoma, a common condition in this type of cancer is a acanthocytosis blood concentration (cells as sea urchin). E: aniso-poikilocytosis ovalocytosis, Kaposi's sarcoma, the predominant pathology was an aniso-poikilocytosis macro-dacryo ovalocytaire (no hollow center) F: ovalocytosis with blood concentration, nasopharyngeal carcinoma, pathology observed CCder a microscopic is anisotropic–poikilocytosis.

(variation of form), microcytosis (cells smaller than normal) on the breast cancer (Figure 2A). In the case of intra uterine neoplasia, pathology observed was a dacryocytosis (cell in tear, Figure 2B). Pathology observed on the rectum cancer was a macro-dacryocytosis and aniso-poikilocytosis hypochromic (Figure 2C). In the case of the liver cellular carcinoma we saw acanthocytosis (cells in form of sea urchin, Figure 2D). We observed in the case of sarcoma of Kaposi, a predominant pathology as macro-dacryo-ovalocytosis (cells with hallow in center) was a macro-spherocytosis with blood concentration (Figure 2).

Data obtained on averages and standard variations of various blood parameters showed that the red blood cells rate between the various types of cancer once opposed to control was significant ($P < 0.0001$). When we compared the rate of hemoglobin's of control with that of various solid cancers, we observed that rate of hemoglobin with that of various types of solid cancer decreased. According to this parameter, results obtained by comparing parameters obtained in solid cancer patients with those of control were significant (Table 2). Distribution of various pathologies showed hypoglobinemia as the most dominating pathology (30.76%) and blood concentration (18.75%). This result showed the hypochromic character of blood tissue and the presence of true anemia (Figure 3).

Cytometry analysis is posted in the form of diffractional diagram in which the neutrophils are represented in green; lymphocytes in blue; monocytes purple and the basophilic ones in white. In the case of breast cancer we noted a great in the absence of eosinophils granules, and varied cells form which could oscillate between size 50 and cuts it 250 units (reading made on the y-axis). Below the bar of separation of reference mark (bars white which cuts the diagram), one observes the neutrophils (in yellow) with a good granularity with a size of oscillating core between 100 and 250 units (observation along the x-axis) (Figure 4A).

In the case of uterine neoplasia, the eosinophilic granules is almost missed while the granules neutrophils are abundant with the varied core shape (Figure 4B). For the cavum cancer; we noted a weak presence of eosinophilic granules and the granulocytes eosinophilic present had various sizes. The granules of the neutrophils in yellow are well present and compact between 50 and 200 units (Figure 4C). In the case of Kaposi sarcoma (Figure 4D) we noticed a strong presence of neutrophils and a beginning of the appearance of lymphocytes (in blue) and the population of eosinophilic is in trace. In liver cancer (Figure 4E), a presence of eosinophilic granules which is in great

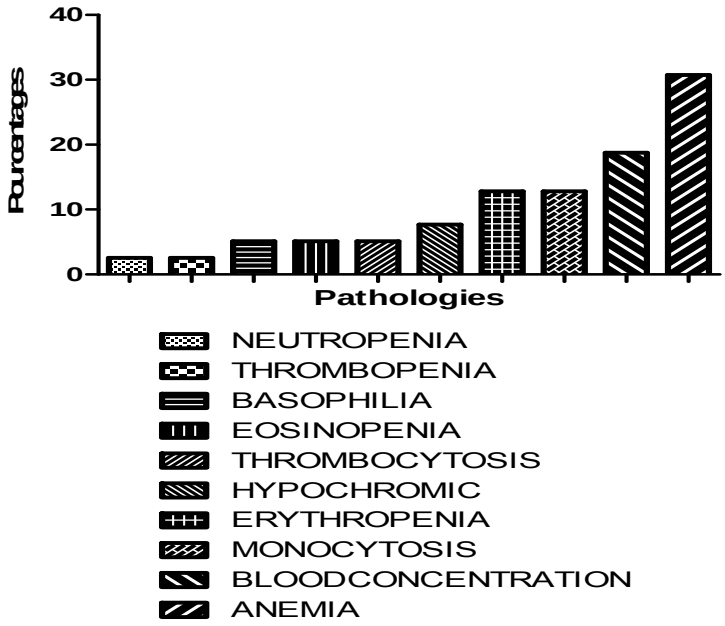

Figure 3. Various pathologies of numeration formulate blood. Distribution of various pathologies shows that the hypoglobinemia is the most dominating pathology (30,76%). Bloodconcentration (18, 75%) shows the hypochromic character of blood tissue and present true anaemia. Monocytosis accounts for 12, 82% of pathologies of blood tissue, an equal incidence with the erythropenia. thrombopénia and neutropenia are met than all other blood pathologies and account for approximately 2.56%.

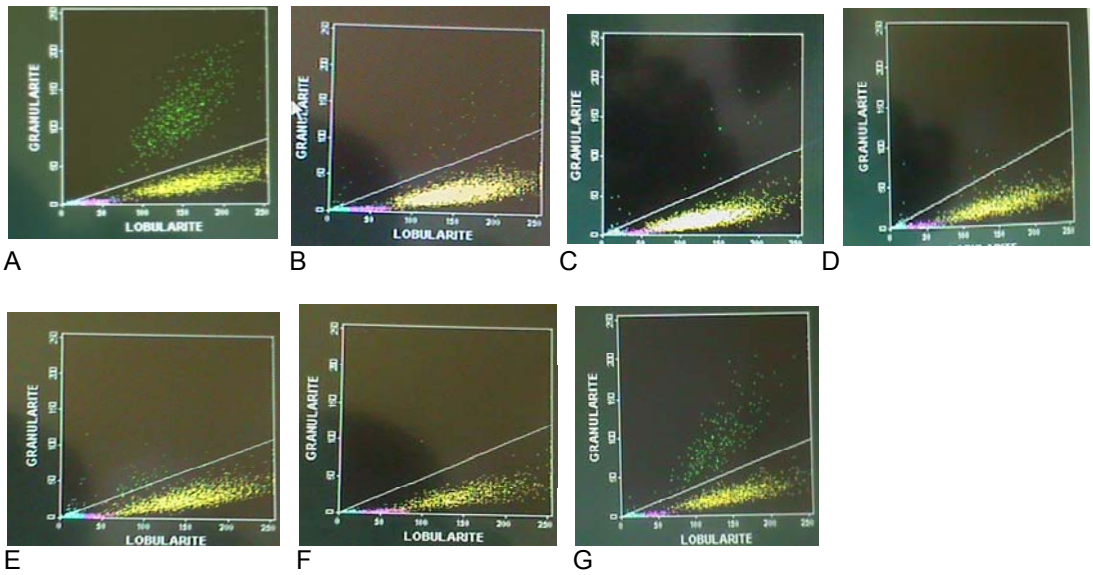

Figure 4. Flow cytometry analysis results. A: breast cancer, presence of eosinophilic granules. B:cancer of the cervix; weak distribution of eosinophilic, in term of granularity and strong concentration of neutrophils. C: cancer of the cavum;large a agranulocytosis eosinophilic disproportionate. D: Kaposi's cancer thorough eosinophilic Agranulocytosis. E: cancer of the liver; one observes a hypo distribution of eosinophilic and neutrophils. F: cancer of the rectum. The intermingling of eosinophilic with the neutrophils in rate of granules and the shape of the nuclei. G:control; good distribution of the granularity and the lobularities, rate of eosinophilic and neutrophils supposed normal being.

Table 2. Blood count results.

Parameter	Controls (n=30)	Cancers types (n = 6)							
		MAC (n = 38)		CHC (n = 10)		KS (n = 8)		NU (n = 26)	
		Mean ± standard error	P	Mean ± standard error	P	Mean ± standard error	P	Mean ± standard error	P
GR	*5.1±0.1	4.4±0.1	0.0001s	4.4±0.2	0.0062s	4.4±0.2	0.00030s	4±0.2	< 0.0001s
HGB	15±0.2	10.9±0.2	< 0.0001s	14.4±0.2	< 0.0001s	14.4±0.2	< 0.0001s	10±0.2	< 0.0001s
PLT	358.7±15	231.3±11	< 0.0001s	236.3±40.2	0.0010s	220.3±17.5	< 0.0001s	194±7.7	< 0.0001s
GB	7.76±0.3	5.23±0.2	0.0002s	4.6±0.5	< 0.0001s	4.5±0.2	0.009s	5±0.4	0.0002s
NEU	39.10±1.7	45.88±1.7	0.0039s	51.45±2.7	0.0010s	48.65±1.8	0.0002s	48.92±2.5	0.0024s
EOS	0.28± 0.03	0.44±0.03	0.0023s	0.37±0.08	0.1213ns	0.41±0.04	0.0113s	0.29±0.07	0.4603s
BAS	0.01±0.0	0.09±0.02	0.0024s	0.03±0.00	0.0128s	0.02±0.00	0.0049s	0.04±0.01	0,0035s
LYM	3.26±0.1	4.9±0.26	< 0.0001s	4.37±0.4	0.0147s	4.9±0.3	0.0002s	6.1±0.5	< 0.0001s
MON	0.4±0.03	0.7±0.16	0.0498s	0.8±0.26	0.0220s	0.53±0.1	0.2081ns	0.6±0.1	0.1030ns

RC: rectal cancer. KS: Kaposi's sarcoma. HCC: hepatocellular carcinoma. NPC: nasopharyngeal carcinoma. CC: cervix cancer. MAC: mammary adenocarcinoma. * = mean ± standard error. ns = not significant, s = significant, GR: red blood cells HGB: hemoglobin; PLT: plate, GB: white blood cell; NEU: neutrophils; EOS: eosinophilic; BAS: basophils; LYM: lymphocytes, MON: monocytes.

quantity (until size 200 on the axis of ordered) have been noticed. The eosinophilic granules are also in great quantity and a small family of lymphocyte is also observed. We also observed an intermingling of eosinophilic (Figure 4F) with the neutrophils in rate of granules and the shape of the cores (Figure 4).

DISCUSSION

Microscopic analysis of May-Grün- wald-Giemsa (MGG) stained slides revealed microcytic poïkilocytosis in breast adenocarcinoma. These erythrocytes morphologies are observed in most individuals affected by this cancer type and were different from those of the control, with normal cells. This observed microcytosis can be partly explained by iron deficiency (low serum iron) which is the main feature of this microcytosis as described by Ankri et al. (2005). Iron deficiencies can be explained by haemorrhages probably caused by alteration cancer forms that are related to internal structural factors of erythrocyte cytoskeleton (Degenne and Binet, 2009). The dacryocytosis observed in neoplasia intrauterine can be explained by abnormal erythropoesis (Méric and Morère, 2005). Milano in 2005, attributed erythropoiesis dysregulation to production and abnormal use of the erythropoietin (Milano, 2005). Cancer of the rectum of these samples presented microcytic aniso-poïkilocytosis. These results corroborate with those of Vignot and Spano (2005), which show that microcytosis are often caused by anemia associated with chronic colorectal cancer, microcytic low iron, due to a tumor (VS and Spano, 2005). An inflammatory component can also take part in its installation particularly, in event of tumor from the metastatic start of necrosis or infection. Hepatocellular carcinoma show acanthocytosis generally observed fibrosis and cirrhosis (Atul and Victor, 2010). Cytological deteriorations are met in almost all types of cancer studied (Robby et al., 2012); some would be directly related to the situation of the state of cancer patients while others can be due to other factors. In general, cytological analyses were shown to be a good tool for diagnosis of blood pathologies induced by solid cancers. Hematological analyses were carried out in order to highlight the quantitative variations of blood cell constituents. Results of red globule rate observed at control (5.1 ± 0.1, n = 30) are different from those observed among patients with various types of cancer. Hemoconcentrations accounted for 18.75% of pathologies met. With low values of hematocrite, what can be characterized as false polyglobulies (Ankri et al., 2005) showed that the true polyglobulies can be characterized by an increase in the total globular volume (isotopic measurement of the blood mass) and hematocrite rate of higher than 58%.

On the other hand the polyglobulies distortion would testify to a significant microcytosis. This observation can corroborate with results of cytology of this study which had identified microcytosis in almost all types of cancer. The hypoglobinemia were predominant pathologies

during all this study (30.76%). Indeed they are anemia. Milano (2005) presented anemia like frequent complications among cancer patients resulting either from the disease and its evolution, or of the specific treatments, in particular chemotherapy and the radiotherapy.

In our study, all the patients were free from any anti-cancer therapy, or could then deduce from it that these hypoglobinemia would result from the evolution of the disease. The result of the HCT presented is significant (P = 0, 0002) between cavum cancer and liver-cellular carcinoma, which is not the case with the other types of cancer. The sarcoma of Kaposi seems to be non significant once compared with the cavum cancer. The monocytosis was met among predominant pathologies. This fact is probably related to their viral origin. Gavrilovic et al. (2010) present Epstein-Barr virus (EBV) like one of etiologic factors of the most significant of the appearance of the cancer of the cavum while the HPV can be at the origin of the cervix cancer (Gavrilovic et al., 1996). The monocytosis observed in these two types of cancer would be probably due to an immunizing response of the organization to the viral infection. Liver-cellular carcinoma and rectum cancer were better characterized. The parameters which could be used as specific marker being able to be employed for their exploration were respectively, hypoglobinemia, a normal IAP, a high PCT, a hypochromic, and a macrocytosis. These parameters should however be combined with other techniques of diagnosis for confirmation (cytology for example).

It is noticed that the blood parameters would be quantitatively faded. Some pathology depending on the size of the cells could not be highlighted by the automat such as the microcytosis which was however observed in cytology. This observation has advantages which could exist by twinning cytological results with blood count results to diagnose solid cancer. The internal exploration of blood cells was carried out by analysis through flow cytometer. Results obtained during this analysis came to supplement cytology results and the blood cells count results. The results of the control presented diffractional diagram with the absence of eosinophilic granules (green) and a presence of neutrophils (yellow). This situation could be inherent in the state cancer patients. Sizes of cores also varied in all the results of our sample. This observation was similar to that observed in the individual controls.

The flow cytometry was presented as a technique which can offer multiple possibilities than traditional tests (agregometry). This technique brought information components and thus was deduced from the functional approaches which revealed the significant granules roles and nuclear forms of solid cancer. An optimal control of the cellular internal states would remain pre-necessarily impossible to circumvent with any exploration of this pathology type. The cytometry in flow would offer in addition to many advantages, the forefront on which would appear the sensitivity of the technique and the possibility of working in total blood, on very small samples as observed in this study.

Conclusion

This study consisted in the exploration of blood cells in six types of solid cancer in order to understand the action of solid cancer in blood tissue. It was noticed that the hematological and cytological parameters altered in individuals with non-leukemic cancer. Hematological parameters (the red blood cells, hemoglobin, the rate of polymorphonuclear platelet, globular volume) showed instability in different types of solid cancers. Cytological abnormalities such as changes of shapes and sizes (aniso-poïkilocytosis) permitted to detect a disorder on the cytoskeleton of blood cells (erythrocytes), probably due to the influence of "mediators" of solid cancers. The nuclear form of control (Lobularity) and the number of granule by flow cytometry revealed a decrease in the rate of polymorphonuclear (agranulocytosis), and a variation in the size of the nucleus of the latter.

Conflict of interest

Authors have none to declare.

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