”CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY

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PROGNOSTIC FACTORS AFTER DEBULKING SURGERY FOR ADVANCED STAGE OR RELAPSED OVARIAN CANCER

DOCTORAL THESIS - ABSTRACT

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CONTENT

General aspects

In the first chapter of the thesis I reviewed the latest epidemiology data related to advanced ovarian neoplasm, known to be one of the most aggressive malignancies affecting women worldwide. Even though multiple advances have been made in terms of laboratory diagnosis and imaging exploration of these patients, early stage diagnosis remains a real challenge. As a result, more attention has been paid to the improvement of prevention and screening methods, thus trying to achieve an early diagnosis and, in long term outcomes, a decrease of the mortality caused by this neoplasia [1-3]. Thus, I believe that a good understanding of the mechanisms of occurrence of this neoplasia ensures not only a clear description of its pathogenesis but also discovering new therapeutic alternatives in cases poorly responsive to standard oncosurgical therapy. As for the screening tests and early diagnosis, I have emphasized the fact that, despite the various advances made so far in terms of laboratory tests and imaging exploration, there is still no test that can provide an early detection of this neoplasia, most cases being diagnosed in advanced stages of the disease [5-7].

In the second chapter I analyzed the most commonly incriminated dissemination patterns of ovarian neoplasms, special interest being paid for hematogenous, peritoneal, lymphatic dissemination as well as for dissemination through contiguity. Thus, I believe that a good understanding of these phenomena helps us to identify new prognostic factors and respectively new lines of treatment that target each individual pattern so that we can provide a good control of this neoplasia. It is known that, in advanced stages, even if the principles of debulking surgery and adjuvant systemic treatment are respected, almost two-thirds of cases will develop a recurrence in the first five years from the initial diagnosis, the recurrence occurring on one or more of the channels of dissemination listed above. Consequently, a good understanding of these mechanisms and respectively of the prognostic factors that can influence their occurrence can provide a personalized approach to each patient, thus maximizing the achievement of long-term survival rates.

In the following chapters, I focused my attention on describing the main prognostic factors that seem to influence the immediate and long-term evolution of these patients. Although traditionally it has been considered that the existence of a less advanced initial stage in association with a complete cytoreduction represent the main prognostic factors, more recent studies have demonstrated that the evolution of patients with ovarian cancer is influenced by a multitude of factors related to the inflammatory status , nutritional status

and, perhaps to the greatest extent, by the fluid-coagulant balance of each patient. Starting from these observations, I initially analyzed the impact of sarcopenia, of the number and characteristics of circulating platelets, of the ratio of various elements in the leukocyte formulas (neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio), hyponatremia, volume imbalances (hypovolemia/hypervolemia), nutritional status (defined by serum concentrations of albumin and total proteins but also by the values of various nutritional prognosis scores) as well as the administration of neoadjuvant chemotherapy on the immediate postoperative evolution of patients with advanced stage ovarian cancer. At the end of this chapter, I discussed the role of risk prediction models for severe complications/perioperative mortality [8]. The next aspect that I analyzed was related to the identification of long-term prognostic factors, the analysis at this time starting with the radicality of cytoreduction and continuing with the role of noadjuvant chemotherapy, the influence of the existence of distant lymphatic metastases, preoperative sarcopenia, the number and characteristics of preoperative platelets, preoperative anemia as well as various parameters that describe erythrocytes (such as the width of erythrocyte distribution), hyponatremia, hyperglycemia or hypoalbuminemia. I thus demonstrated that all these parameters seem to influence in a complex manner the long-term evolution of advanced stage ovarian cancer [7].

The last chapter of the general part aimed to emphasize the importance of the multidisciplinary approach and tertiary treatment centers in such cases. Once the concept of multidisciplinary approach is created, the therapeutic plan for patients with suspected ovarian neoplasm will transform from a linear one (with the surgeon, the anatomopathologist and the oncologist as the main links) to a circular one, having the oncosurgeon as well as the medical oncologist, the gynecologist, the vascular surgeon, the urologist, the pathologist, the radiologist, the nuclear medicine doctor, the psychologist and the genetician [8,9].

I. Personal contribution

Working hypothesis and general objectives

As I mentioned in the general part, I started from the hypothesis that in the evolution of patients advanced stage ovarian cancer submitted to debulking surgery with radical intent as first therapeutic option, various prognostic factors are involved, both related to tumor biology (histopathological type and subtype, degree of differentiation) as well as to the patient's clinical condition (translated by nutritional status, associated comorbidities) and respectively by the patient's bio-umoral status (expressed by various parameters – homeostasis, fluid-coagulant balance). The great advantage of these prognostic factors is that they can be easily identified even from the preoperative period and can thus ensure a better selection of patients who will benefit from surgical intervention with curative intent, or in other words, they will allow highlighting of cases in which per-primam surgery would preferably be replaced by the administration of neoadjuvant chemotherapy.

Research methodology

In the present study, we retrospectively analyzed data of patients undergoing surgery with the presumptive diagnosis of advanced or recurrent ovarian cancer between January 1, 2014 and January 1, 2019 in Dr. Ioan Cantacuzino Clinical Hospital, in all cases complete debulking surgery being tempted. The initial selection was made based on the admission diagnoses of these patients, a final number of 97 patients being identified. Later, following the analysis of the histopathological results, seven patients were excluded from the study due to the fact that the final histopathological result was FIGO stage II-IIIB ovarian cancer – in 11 cases, uterine body neoplasm with ovarian invasion in three cases, primitive peritoneal tumors in two cases and respectively of Krukenberg tumor with possible gastric origin in one case. Finally, we identified a number of 75 patients with histopathologically confirmed diagnosis of advanced or recurrent ovarian neoplasms: 57 cases of FIGO stages IIIC-IV ovarian cancer and 18 cases of recurrent ovarian neoplasms. Clinical, biohumoral, imaging, histopathological data as well as data on distant evolution were collected retrospectively.

Statistical analysis

Statistical analysis was performed using SPSS software, version 18.0 (SPSS Inc. Chicago, IL, USA). We used a receiver operating characteristic curve as well as the Youden index [maximum (sensitivity+specificity -1)] in order to estimate the optimal threshold value for various parameters. The initial event was considered the time of primary cytoreduction, subsequently the next events that were taken into account were secondary cytoreduction and time of death, all these events being analyzed until June 1, 2023. The disease-free period was defined as the interval between initial surgery (and end of adjuvant chemotherapy after initial surgery, respectively) and diagnosis of recurrence while the overall survival was determined as the time interval from the time of initial surgery to the time of death.

Univariate analysis was performed using the Cox proportional hazard model. Multivariate analysis was performed using the multivariate Cox proportional hazard model. Median survival for various subgroups was obtained by Kaplan Meyer analysis; subsequently survival curves were compared using the Log-rank test. Statistically significant results were considered those for which the p value is below 0.05. These analyses were performed both in the group of patients subjected to primary cytoreduction (which included 57 cases) as well as in the group of patients submitted to secondary cytoreduction (which included 18 cases).

The influence of preoperative clinical status on the long-term outcomes of patients submitted to primary cytoreduction for advanced stage ovarian cancer

Introduction (working hypothesis and specific objectives)

The preoperative clinical status seems to greatly influence the postoperative evolution of patients with advanced stage ovarian cancer. This status is dictated both by the presence of various associated comorbidities and by the nutritional status of these patients. Starting from the general observations that obesity (translated by a body mass index of more than 30 kg/m2) is frequently associated with the presence of multiple metabolic disorders such as hypertension, dyslipidemia, type II diabetes or sleep apnea, disorders that create an intense pro-inflammatory and pro-carcinogenic environment, we investigated the hypothesis that obese patients diagnosed with ovarian neoplasms are predisposed to a particularly unfavorable evolution.

Material and method

The data of patients with histopathologically confirmed advanced stage ovarian cancer, submitted to primary surgical intervention between 2014-2019 in the Clinical Hospital "Dr. Ion Cantacuzino" were retrospectively reviewed; cases in which the final staging (based on the histopathological analysis) was not IIIC or IV were excluded; we also excluded from the study the cases that underwent neoadjuvant oncological treatment. Nutritional status was assessed by calculating body mass index - BMI - (defined as weight (kg)/height (cm) ^{2}). Thus, patients with BMI <18 kg/m^2 were considered underweight, those with BMI between 18.1 and 24.9 kg/m^2 were considered normal weight, those with BMI between 25 and 29.9 kg/m^2 were considered overweight and those with a BMI over 30 kg/m^2 were considered obese (obesity grade I - BMI < 34.9 kg/m^2, obesity grade II - BMI < 39.9 kg/m^2, obesity grade III - BMI < 44.9 kg/m^2, morbid superobesity – BMI >45 kg/m^2). The nutritional status was also assessed by preoperatively determining the serum values of total serum proteins (a normal value being considered to be over 6.5g/dl) as well as the serum albumin level (hypoalbuminemia being defined as a value of serum albumin below 3.5 g/dl). Postoperative complications were assessed according to the Dindo Clavien scale.

Results

After the analysis of the histopathological results and the exclusion from the study of patients in whom stages IIIC – IV or ovarian neoplasm histology could not be proven, 57 cases were identified. The mean age at the time of surgery was 56.4 years (range 25 to 83 years). Using a cut off of 60 years, the patients were subsequently divided into two groups, those under 60 years of age – 31 cases and respectively those over 60 years of age – 26 cases.

The main signs and symptoms were represented by asthenia, in 51 of the 57 cases, and increased abdominal volume, respectively, in 38 of the 57 cases. Ascites fluid was detected intraoperatively in 51 of the 57 cases, the average amount being 2400 ml (between 200 and 6500 ml). Regarding the preoperative value of CA 125, it was dosed in all cases and varied between 78 and 13500U/ml, with a mean value of 2891 U/ml (the maximum value accepted as normal being 35 U/ml). Regarding the imagistic studies, in all cases the patients were subjected to chest, abdomen and pelvis computer tomography examinations in order to exclude intra-thoracic metastases; in 23 of the 57 cases, there was suspicion of peritoneal carcinomatosis in the computed tomographic examination, which is why the imaging exploration was completed with a nuclear magnetic resonance type examination that confirmed the presence of carcinomatosis lesions. In the remaining 34 cases, there were no imaging signs of secondary peritoneal determinations, but these were objectified at the time of the surgical intervention. Preoperatively, the mean diameter of the peritoneal nodules was 2.2 cm (between 1.5 and 12 cm); it should be mentioned that a diameter of over 7 cm was described in 11 cases, in eight of which it was associated with "omental cake". Furthermore, in terms of resectability, in three of the eight cases omental cake was the cause of unresectability due to the infiltration of small bowel loops located in close proximity to the tumorally-transformed omentum.

The nutritional parameters that were evaluated preoperatively were represented by body mass index (BMI), serum albumin and total protein concentration. A significant problem we faced was represented by the presence of large amount of ascites in some cases, which influenced BMI in absolute value; for this reason, we adjusted the weight of the patients according to the ascites volume estimated at the CT scan. Thus, the average BMI value was 26.8 kg/m2, between 17.5 and 43 kg/m2. Regarding the distribution by weight class, there were 11 underweight patients (BMI below 18 kg/m2), 19 normal weight patients (BMI between 18.1 and 24.9 kg/m2), 13 overweight patients (BMI between 25 and 29.9 kg/m2), eight patients with class I obesity (BMI between 30 and 34.9 kg/m2), three patients with class II obesity (BMI

between 35 and 39.9 kg/m2) as well as three patients with morbid obesity (BMI over 40 kg/m2). As for the preoperative serum values of alumin and total proteins, they ranged between 1.8 and 4.4, with a mean value of 2 g/dl, and between 3.6 and 8.2, respectively, with a mean value of 5, 8 g/dl. Later, these determinations were used both in this form and in the form of various nutritional scores in order to evaluate the influence of nutritional parameters on immediate and long term outcomes.

Regarding the type of surgery, it standardly consisted of total hysterectomy with bilateral adnexectomy, omentectomy, and pelvic lymph node dissection; various other surgical gestures were added to this in order to achieve maximal cytoreduction.

Thus, the associated interventions were represented by rectosigmoid resections in nine cases, right hemicolectomy in five cases, subtotal colectomy in four cases, splenectomy in seven cases, appendectomy in three cases, partial cystectomy with cystoraphy in four cases, partial cystectomy with left distal ureterectomy and ureteral reimplantation in two cases, atypical hepatectomy in four cases, aortocaval lymph node dissection – in 31 cases, pelvic peritonectomy – in 27 cases, parietal peritonectomy – in 22 cases, diaphragmatic peritonectomy – in 14 cases, partial diaphragmatic resection with phrenorrhaphy – in four cases, cholecystectomy in three cases, segmental entrectomy with anastomosis in three cases and respectively with terminal ileostomy in two cases, distal pancreatectomy – one case and gastric resections respectively – three cases. It should be noted that in nine of the 57 cases there were disseminated tumoral nodules at the level of the mesentery for which electrofulguration was performed.

In all cases maximal cytoreduction was tempted; however R0 resection was feasible in 47 of the 57 cases, while in the remaining 10 cases an incomplete debulking was performed: R1 resection was feasible in four cases while R2 resection was performed in six cases respectively. In 11 of the 57 cases, cytoreduction had been also attempted in other units, but at that time the intervention was limited to biopsy; subsequently, in seven of the 11 cases, the patients underwent neoadjuvant chemotherapy followed by interval debulking, while in the other four cases, the patients underwent a new debulking attempt. Maximal debulking was achieved in five of the seven cases undergoing neoadjuvant chemotherapy and in one case that was directly reoperated.

Regarding the correlation between age and the feasibility of maximal debulking, we found that it was obtained in 28 cases under 60 years of age and respectively in 18 cases over 60 years of age, this correlation not having statistical significance (p= 0.08).

Regarding the intraoperative details, the mean duration of the surgical interventions was 190 minutes (range 70 to 320 minutes) while the mean blood loss was 550 ml (range 100 to 2300 ml); with regard to the transfusion requirement, 18 of the 57 patients required the administration of erythrocyte meals intraoperatively, the average number being 2 meals (between 1 and 4 erythrocyte meals)

Early postoperative outcomes

The early postoperative course was assessed by investigating morbidity and mortality in the first postoperative month. Thus, 17 of the 57 patients (29.8%) suffered postoperative complications within the first postoperative month, three of them dying in the first 30 days (5.2%). It should be noted that there was no intraoperative death while the average duration of hospital in stay was of 10 days (between 3 and 37 days). Postoperative complications were as follows:

- Grade I complications:
• wound infections or blocked eviscerations - 2 cases, both were treated conservatively
• ileus – 1 case - conservative management
- Grade II complications:
• Pneumonia – 1 case – required prolonged antibiotic treatment, did not require admission to intensive care nor other invasive maneuvers
• Deep venous thrombosis – 2 cases – required the addition of anticoagulant treatment by doubling the doses of enoxaparin and prolonging this treatment 30 days after discharge; subsequently up to 6 months postoperatively the patients received oral anticoagulant treatment
• Colonic anastomotic leaks – 2 cases, conservative management
• Acute hemorrhagic gastritis – 1 case – required the continuous administration of proton pump inhibitors
• Pelvic hematoma – 1 case – conservative management – repeated transfusions, broad-spectrum antibiotic therapy to avoid hematoma infection
- Grade III complications:
• Subphrenic abscess that required percutaneous drainage – 1 case. The patient also associated a right pleurisy, which was also drained percutaneously.
- Grade IV complications:

• Hemoperitoneum – 2 cases, 1 of them required hemostatic dressing with re-intervention for decompression 48 hours postoperatively.

• Uroperitoneum after partial cystectomy with cystoraphy - re-intervention on the 5th postoperative day - favorable evolution

- Grade V complications

• Perforated gastric ulcer – acute generalized peritonitis – re-intervention on the 10th postoperative day – death

• Hemoperitoneum at 12 hours postoperatively – it required hemostatic dressing followed by subsequent decompression at 48 hours, followed by the appearance of tonico-clonic convulsions (the patient being known to have epilepsy) and death 24 hours after reintervention

• Colonic anastomotic leak – reintervention on the 7th postoperative day, multiple organ dysfunction, death on the 10th day after the initial intervention.

When analyzing the distribution of complications by age group, we noticed that they were reported in eight cases under the age of 60 and respectively in 8 cases over the age of 60 ($p=0.771$); however, severe complications (Dindo Clavien grade of more than 3) requiring reoperation were encountered in only one case under 60 years of age and in five cases over 60 years of age, respectively. Moreover, three of the five patients over 60 years of age who developed severe complications died during the first postoperative month, while no deaths occurred in the group of patients under 60 years of age.

The histopathological results were: serous adenocarcinoma in 35 cases, clear cell carcinoma in 7 cases, endometrioid adenocarcinoma in 5 cases, mucinous adenocarcinoma in 5 cases, carcinosarcoma in 2 cases, dysgerminoma in 2 cases and clear cell endometrioid carcinoma respectively in 1 case. Regarding the degrees of differentiation, there were six cases of well-differentiated tumors, 15 cases of medium differentiation and 36 cases of poorly differentiated tumors. When analyzing the subgroup of patients who developed postoperative complications, I noticed that they were significantly older compared to those in whom the evolution was uncomplicated (68 versus 53 years, $p=0.0002$) and presented at least two associated comorbidities. As expected, patients in whom more than two visceral resections were needed (in association with the standard intervention of total hysterectomy with bilateral adnexectomy and omentectomy) developed postoperative complications more frequently ($p=0.002$) when compared to cases in whom the surgical procedure was limited to total hysterectomy with bilateral adnexectomy and omentectomy; surprisingly, the location of the resected viscera (upper abdomen versus lower abdomen) did not influence the risk of postoperative complications. Thus, complications occurred in 12 of the 40 patients who benefited from extensive resections also at the level of the upper abdomen and respectively in

4 of the 17 cases in which the resection was limited to the pelvic level (p=0.7529). I also observed that patients who developed postoperative complications had a significantly higher BMI compared to those whose evolution was uncomplicated (37.5 kg/m2 versus 23.6 kg/m2 , p=0.002) and respectively a serum level of albumin and total proteins significantly lower (1.8 g/dl versus 3.7 g/dl and respectively 4.1 versus 7.3 g/dl, p=0.001 and respectively p=0.0002).

As for the possible correlation between the existence of comorbidities and the extension of cytoreduction in the upper abdomen, comorbidities were present in 13 of the 40 patients in whom the cytoreduction extended to the upper abdomen and respectively in 9 of the 17 patients in whom the resection was limited to the lower abdomen (p=0.2338). In conclusion, the presence of preoperative comorbidities did not limit the extension of debulking surgery in the upper abdomen in a statistically significant manner. Regarding the duration of hospital in stay, the average interval was of 7 days for the cases in which the evolution was simple and 23 days respectively for the cases that developed postoperative complications.

Conclusions

The preoperative clinical status seems to significantly influence the postoperative evolution of patients diagnosed with advanced stage ovarian cancer. Thus, most complications are expected among elderly, obese patients who have other associated comorbidities and who have a low level of circulating albumins and proteins. While the age factor cannot be changed, the others can be at least partially corrected. As a result, amelioration or correction of comorbidities or the administration of enteral or parenteral hyperprotein nutrition seem to decrease the risk of immediate postoperative complications. Once the immediate postoperative evolution of these patients is uncomplicated, they will benefit more rapidly from the administration of adjuvant oncological treatment, thus decreasing the risk of recurrence and therefore increasing the hope of long-term survival.

The influence of hyponatremia on the early and long term evolution of patients diagnosed with advanced stage ovarian cancers undergoing primary cytoreduction

Introduction (study hypothesis)

Hyponatremia represents a frequently encountered hydro-electrolyte disorder in patients diagnosed with advanced ovarian neoplasms, its presence being explained by numerous mechanisms such as excessive vasopressin secretion by tumor cells, dehydration and loss of electrolytes through diarrhea or vomiting, inability to feed and hydrate properly , the presence of intravascular fluid extravasation in space III (ascites) or even due to the administration of various treatments (anti-inflammatory, diuretic or chemotherapy). In this context, it can be easily understood why a large part of patients diagnosed with ovarian neoplasms present at least at some point in their evolution an episode of hyponatremia. Considering all the above stated mechanisms that can lead to the occurrence of this dyselectrolytemia, I investigated whether the presence of hyponatremia can be considered as a bad prognostic factor both for the immediate and long-term postoperative evolution of patients diagnosed with ovarian neoplasms. I also investigated whether the preoperative presence of hyponatremia is associated with a higher risk of incomplete debulking, and therefore with the preoperative identification of patients with advanced stage ovarian cancer who would benefit from neoadjuvant chemotherapy rather than per primam surgery.

Material and methods

The data of the 57 patients who underwent debulking surgery between 2014 and 2020 were retrospectively analyzed. In all cases, the preoperative natrium value was determined 24 hours before the surgical procedure, hyponatremia being defined as a value of serum sodium below 135 mEq/l. Also, before the introduction of the patients into the study, a thorough anamnesis was aimed at excluding the administration of systemic treatments that have the effect of changing systemic sodium (such as some classes of diuretics, some oral antidiabetics or certain classes of anti-inflammatories). The Cox hazard model was used to identify factors with statistical significance; once identified, they were entered into the multivariate analysis, the statistically significant difference being considered in the univariate analysis as well as in

the multivariate one if a p value below 0.05 was obtained. Analysis and survival curves were obtained and compared by the Kaplan Meyer method.

Results

The mean value of preoperative serum sodium concentration was 133 mEq/l; 36 cases presented preoperative hyponatremia while the remaining 21 cases presented normal serum sodium values. The mean age of patients with normal preoperative sodium levels was 52.85 years while the mean age of those with hyponatremia was 58.47 years (p=0.001). As for the correlation between the associated comorbidities and the preoperative sodium level, 12 of the 35 cases that did not present associated comorbidities and respectively 9 of the 22 cases that had associated comorbidities presented a normal preoperative sodium level.

Regarding the ascites volume determined intraoperatively, it was significantly higher in patients with hyponatremia (1633 ml versus 490 ml, p=0.0001). As for the correlation between the histopathological type and the serum sodium level, a statistically significant correlation could not be established this time either (p=0.512). Details of the preoperative serum level and the various histological subtypes are represented in figure 9.1.

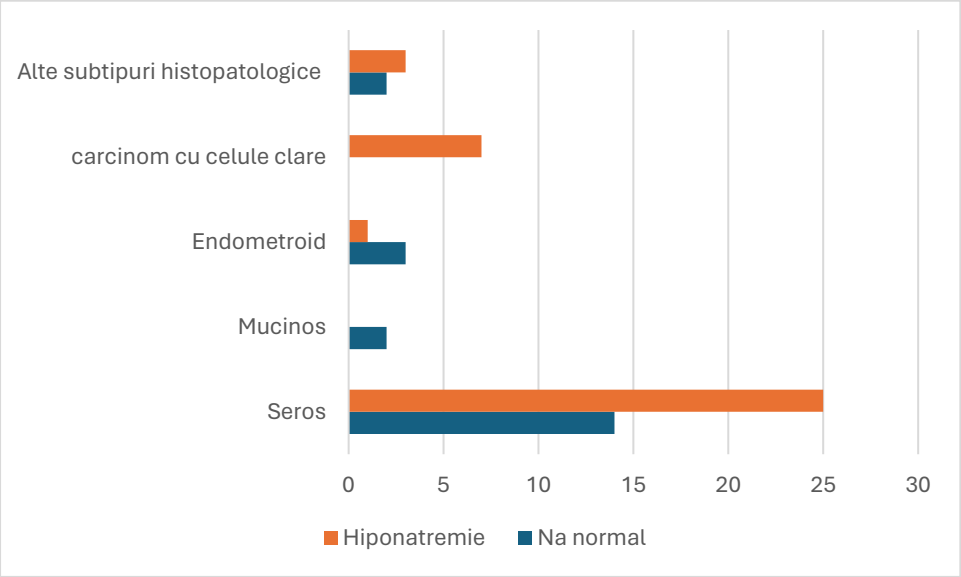

Figure 1: the distribution of the 57 cases accordingly to the preoperative value of natrium and to the histopathological subtype.

When it comes to the relationship between the other bioumoral data and the preoperative natrium levels, this is presented in the table below.

Table 1: the corelation ship between the preoperative levels of natrium and other preoperative parameters determined 24 hours before surgery

	Hiponatremia	Normal serum values of Na	p
No of cases	36	21	-
Mean age (years)	58,47	52,85	0,001
Mean CA125 (U/ml)	4252	561	0,0001
Hb (g/dl)	11,4	12,7	0,057
Albumin (g/dl)	3,6	4,09	0,001
Total serum proteins (g/dl)	5,4	7,6	0,001
Lymphocytes	1193	1947	0,001
Monocytes	782	403	0,001
Thrombocytes (/microl)	415582	285411	0,001
Neutrophils	5990	3780	0,01
LMR	1,85	5,31	0,001
PLR	408	162	0,002
NLR	5,5	2,05	0,001
SII	2431449	602143	< 0,00001
CRP (mg/l)	2,33	17,69	< 0,00001
CRP/albumin (mg/l)	0,08	0,61	< 0,00001
BRCA status - Negative - Positive	 27 9	 2 19	< 0,00001

The next performed analysis was the one between the preoperative serum level of natrium and intra and postoperative details. The results are presented in table 2.

Table 2: the corelation ship between preoperative natrium values and the perioperative outcomes

	Hyponatremia	Normal sodium levels	p
Number of cases	36	21	-
Upper abdominal resections - Yes - No	 25 11	 15 6	1
PCI (mean value)	28	12	0,003

Type of cytoreduction: - Complete - Incomplete	 26 10	 20 1	 0,0047
Postoperative complications	15	1	0,0025
Mean blood loss	890	1100	0,08
Administration of erythrocyte meals (mean)	2	3	0,45
Time of surgery (mean, minutes)	320	380	0,05
Mean hospital in stay	13,48	7,5	0,03

Regarding the long-term evolution of these patients, I observed that patients who presented with low serum sodium levels preoperatively reported a disease-free survival of 10.8 months and an overall survival of 18.5 months, respectively, while cases with normal preoperative sodium levels reported a disease-free survival of 31.4 months and an overall survival of 49.7 months, respectively, in both cases the difference being a statistically significant one ($p<0.0001$ and $p=0.0001$, respectively).

Conclusions

Hypoantremia represents a significant factor of unfavorable prognosis for both short term and long term outcomes in advanced stage ovarian cancer patients. Thus, in cases in which a low sodium level is identified preoperatively, it is expected that the extension of the neopalsic lesions to impede the achievement of maximal debulking.

The influence of the procoagulant status on the early and long term outcomes of advanced stage ovarian cancer

Introduction (working hypothesis and specific objectives)

In the last decades, increased attention has been paid to the research of possible correlations between the coagulant status of patients and the risk of occurrence and extension of different neoplastic procedures. Thus, in addition to the positive roles that platelets have in protecting vascular integrity and preventing haemorrhages, they also seem to actively participate in the body's immune and inflammatory response and respectively to be correlated with the extension of various neoplastic processes. Thus, an increased number of platelets appears to exist in the circulation of many patients for a period of time before they are diagnosed with various malignancies. The presence of tumor cells seems to lead to the secretion of an increased amount of interleukins such as IL6, IL3, IL1 and, respectively, granulocyte-macrophage colony-stimulating factors, which will in turn lead to the appearance of an increased number of circulating platelets and, respectively, monocytes. Once thrombocytosis is induced, it appears to have a stimulating effect on the dissemination and metastasis of tumor cells. Starting from these observations, in the present chapter we studied the possible correlations between the number of circulating platelets and the immediate and distant evolution of patients diagnosed with advanced ovarian neoplasms, the objective being to demonstrate the existence of an inversely proportional relationship between the number of circulating platelets and long-term survival of these patients. A minor objective was to assess the existence of a possible correlation between circulating platelet count and the risk of immediate postoperative complications.

Material and methods

And this time we performed a retrospective analysis of the 57 patients who underwent debulking surgery in the Clinical Hospital "Dr. I. Cantacuzino" between 2014-2020, in all cases taking into account the number of platelets before the surgical intervention. Also, before introducing the patients into the study, we researched the personal pathological antecedents of the patients, with the aim of avoiding the inclusion in the study of some cases in which a possible thrombocytosis could have other causes: the antecedents of splenectomy, the antecedents of hematological diseases or the administration long-term use of treatments that could have thrombocytosis as a side effect. At the time of the primary cytoreduction, the ROC curve (receiver operating characteristic) and respectively the Youden index [maximum

(sensitivity+specificity-1)] were used to obtain the cut-off value of the number of platelets that would have the ability to predict the achievement of maximum cytoreduction. Thus, the cut-off value was set at 335,000, having a sensitivity of 0.801 and a specificity of 0.771.

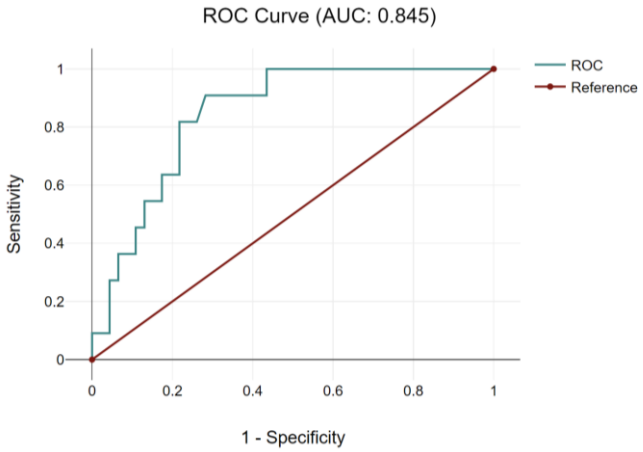

Figure 2: ROC curve obtained to determine the cut-off value of the number of platelets predicting the achievement of maximal debulking - the cut-off value was set at 335,000/microl, with sensitivity of 0.801, specificity of 0.771 and area under the curve of 0.845.

Results

At the time of primary cytoreduction, the mean circulating platelet count was 367,624/microl (range 167,000 – 848,100/microl). Using the cut-off value of 335,000/microl, the group was divided into two subgroups: the group of patients in whom the preoperative value of circulating platelets was below 335,000 – 30 cases and respectively those in whom the preoperative platelet count was over 335,000 – 27 cases. Preoperative and intraoperative characteristics according to preoperative platelet count are shown in the table below.

Table 3: the correlation ship between different preoperative and intraoperative parameters and then preoperative number of platelets at the time of primary cytoreduction

Number of cases	Number of circulating platelets		p
	< 335.000/microl (30 cases)	>335.000/microl (27 cases)	
Age: <60 years >60 years	 18 12	 13 14	 0,43
Histopathological subtype: - Serous	 20	 15	 0,78

- Mucinous - Endometroid - Clear cell carcinoma - Other subtypes	2 4 0 4	3 1 7 1	
Differentiation degree: - G1 - G2 - G3	 5 12 13	 1 3 23	 0,33
PCI: <10 10 -15 >15	 12 13 5	 2 8 17	 0,001
Ascites – mean volume - ml	1566	3222	0,0002
Hemoglobin (g/dl)	12,5	11,1	0,1
Albumin (g/dl) <3,5 >3,5	 13 17	 18 9	 0,11
Comorbidities - Yes - No	 11 19	 11 16	 0,79
Type of cytoreduction: - Complete - Incomplete	 29 1	 17 10	0,0017
Postoperative complications: - Yes - No	 5 25	 11 16	0,07
Total number of cases	30	27	

The average number of circulating platelets at the time of primary cytoreduction among patients in whom maximal debulking was successfully performed was 383,000/microl, significantly lower compared to those in whom debulking was submaximal – 525,000/microl, p= 0.002. As expected, patients with lower platelet count had more frequently a lower peritoneal carcinomatosis index and significantly lower ascites volume compared to those with higher preoperative platelet counts. As for other possible correlations, no statistically significant difference was demonstrated between the frequency of comorbidities or

postoperative complications and the preoperative platelet count and respectively between the preoperative platelet count and age, type and degree of histopathological differentiation, serum albumin or hemoglobin level.

The positive correlation between the preoperative platelet count and the volume of ascites, the extent of the peritoneal carcinomatosis lesions and the type of cytoreduction, respectively, was reflected in a statistically significant manner on the long-term evolution of this group of patients.

Analysis of overall survival demonstrated a significant benefit among patients whose preoperative platelet count was below 335,000/microl. Thus, they reported a median survival of 21 months while this value was only 10 months for cases in which the preoperative circulating platelet count was over 335,000/microl – 21 months, p=0.002 (Figure 3). This benefit was also demonstrated in terms of disease-free survival (Figure 4).

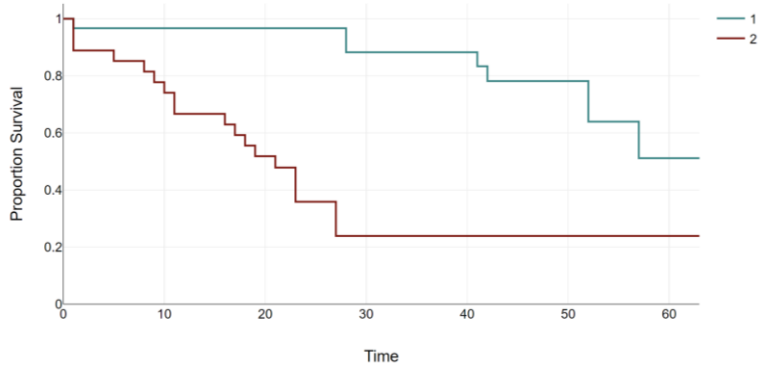

Figure 3: Kaplan Meyer survival curves for patients with a preoperative platelet value below 335,000/microl (group 1) and respectively above 335,000/microl (group 2) demonstrating a significant benefit in terms of survival favouring the first group - 21 months versus 10 months, p= 0.002.

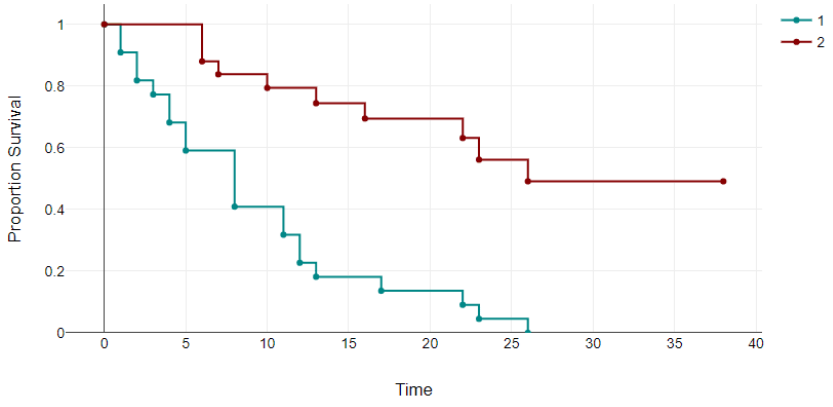

Figure 4: Kaplan Meyer curves of disease-free survival for patients with a preoperative platelet value below 335,000/microl (group 1) and respectively above 335,000/microl (group 2) again demonstrating a significant benefit favoring the first group - 18 months versus 9 months, p=0.001

Conclusions

Patients with advanced stage ovarian cancer seem to have a marked procoagulant status, this being demonstrated by the existence of an increased number of circulating platelets, the severity of the prognosis being directly proportionally correlated to the number of circulating platelets. The exact mechanisms are not known but are suspected to be related to the existence of circulating interleukins and growth factors. Once the pathogenesis of these mechanisms is understood, the premises are created for the use of new therapeutic lines, addressed both to circulating interleukins and to growth factors and, respectively, platelets themselves.

The influence of proinflammatory status on the immediate and distant evolution of advanced ovarian neoplasm

Introduction (working hypothesis and specific objectives)

Given that, despite the development of imaging techniques, a large number of ovarian neoplasms are directly diagnosed at advanced stages, researchers' attention has been focused on investigating if preoperative investigations can provide information on the stage and extent of various neoplastic processes with ovarian origin. Thus, starting from the observation that the existence of a state of chronic inflammation can predispose to the appearance and acceleration of the oncogenetic process, in the present work we investigated the existence of a possible correlation between the components of the hemogram that are responsible for the inflammatory status and the extension of the neoplastic process on a study group of 57 patients undergoing primary cytoreduction during 2014-2020. Thus, we hypothesised that the existence of a large number of cells synthesizing cytokines and tumor growth factors such as neutrophils or monocytes in association with a low number of circulating lymphocytes (which have a well-known role in the defence against neoplastic dissemination) can represent a prognostic factor for advanced stage ovarian neoplasms; moreover, we investigated whether these parameters influence both immediate and long-term postoperative evolution.

Material and methods

The inclusion criteria were: age over 18 years, final histopathologically confirmed diagnosis of FIGO stages IIIc-IV ovarian cancers, epithelial histology of ovarian neoplasia and surgical intervention as the first therapeutic gesture. Patients under the age of 18, with histologies other than epithelial ones, in whom the pathological anatomy could not confirm at least stage IIIC disease, who had undergone neoadjuvant chemotherapy as well as those with a history of autoimmune or hematological diseases were also excluded from this study. In all cases, data regarding the hemograms before the surgical procedure were retrospectively reviewed, as well as data regarding the serum level of CA 125, albumin and C-reactive protein, respectively. When it comes to the radicality of cytoreduction, it was considered complete if there was no visible residual tumor volume at the time of surgery and incomplete if there was macroscopically visible residual tumor volume at the end of surgery. Complications occurring in the first postoperative month were considered as early complications and were classified according to the Dindo-Clavien scale. In order to obtain a threshold value that could have a positive predictive value on the possibility of obtaining maximum debulking surgery, the

Receiver Operating Characteristic (ROC) and respectively the Youden index [maximum (sensitivity + specificity-1)] was used.

Thus, the main investigated parameters were the systemic inflammatory index (systemic inflammatory index - SII) defined as the ratio between the product of platelets x neutrophils and respectively circulating lymphocytes (platelets x neutrophils / lymphocytes), the neutrophil/lymphocyte ratio (neutrophil/lymphocyte ratio - NLR) , the monocyte/lymphocyte ratio (monocyte/lymphocyte ratio - MLR) and the platelet/lymphocyte ratio (PLR), respectively. The AUC for the parameters cited above are shown in the figure below.

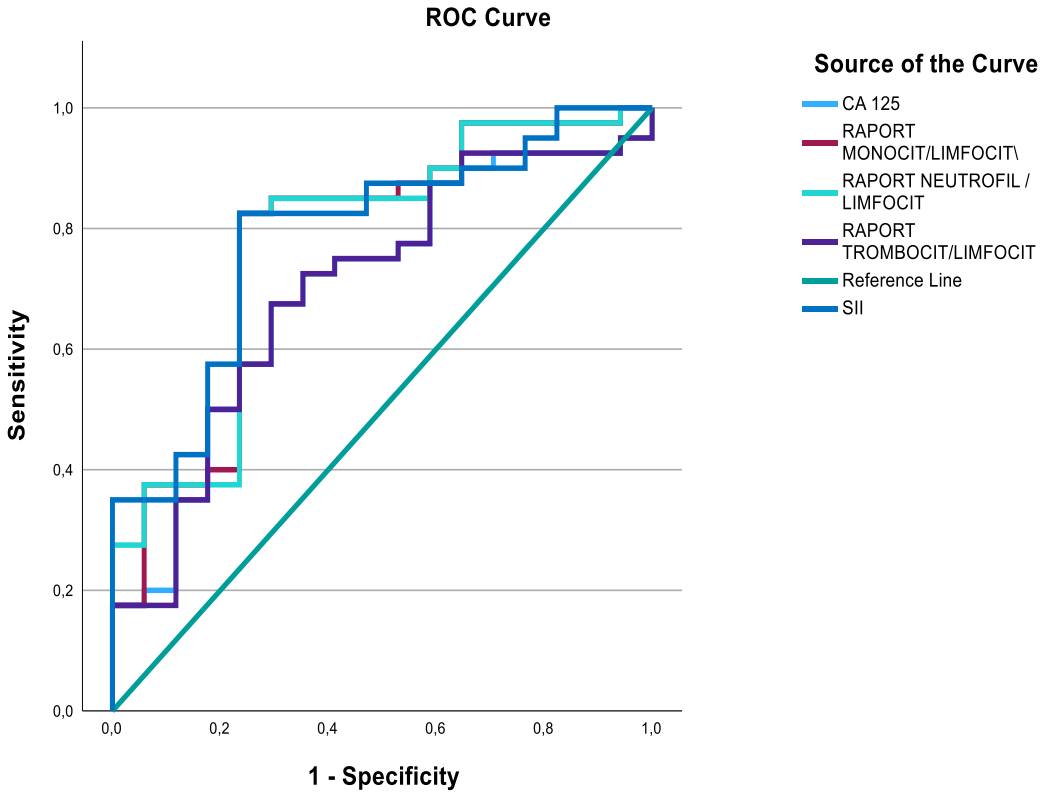

Figure 5: ROC si AUC for CA125, MLR, NLR, PLR, SII

Results

The demographic data of the group of 57 patients were presented in previous chapters, the average age being 56 years (between 25-83 years); taking into account a cut-off value of 60 years of age, this was correlated with significantly more comorbidities among elderly patients compared to those under 60 years of age (17 of the 26 over 60 years of age and 5 respectively of the 31 cases under the age of 60 with such associated diseases, $p<0.0001$). As for the age influence on the type of cytoreduction, no significant correlation could be demonstrated between the two parameters, although complete debulking was more common

among patients under 60 years of age (p=0.08). As for early postoperative complications, although they had comparable frequencies between the two age groups, when analysing only severe complications (defined as having a Dindo Clavien grade of more than 3), they were significantly more frequent among elderly patients: moreover, all three deaths that were reported in the studied group in the first postoperative month were registered in elderly patients.

These data demonstrate that although age itself did not influence the radicality of cytoreductions, the association of severe comorbidities at ages over 60 years seems to decisively influence the postoperative evolution of these patients, possibly leading to their death in the first postoperative month. As a result, we are justified in concluding that the existence of severe comorbidities and not age per se should dictate the extent of surgical interventions.

Later we analysed the bio-umoral information for each individual case and demonstrated that the average value was 11.8 g/dl (between 7.8 -16.1 g/dl) for hemoglobin, 367624/ microl (between 167000-848100/microl) for platelets, 5176/microl (between 1475-12980/microl) for neutrophils, 1471/microl (between 472-3120/microl) for lymphocytes, 643/microl (between 138-1520/microl) for monocytes and 1757495 (between 204265-7548167) for SII.

Using the cut-off values determined above, we divided the patients into two groups as follows: 20 patients with the SII value below 841,000 and respectively 37 patients with the SII value above 841,000, 17 patients with the NLR value below 2.7 and respectively 40 with NLR over 2.7, 21 cases with the MLR value below 0.25 and respectively 36 cases with the MLR value above this threshold, 23 cases with the PLR value below 200 and respectively 34 cases with PLR above 200 and 25 cases with the value CA 125 below 780U/l and respectively 32 cases with CA125 value higher than 780 U/l. At the time of the survival analysis, we demonstrated that patients diagnosed with ovarian neoplasms with a high proinflammatory status consistently reported a significantly worse long-term outcome compared to those with a lower level of systemic inflammation, this evolution being translated into a shorter disease-free and overall survival respectively.

Thus, patients with increased values of CA 125 reported an average survival of 16.97 months, while those with values below 780U/l reported an average survival of 43.61 months, p=0.001, those with increased values of MLR had an average survival of 15.62 months, significantly lower compared to those who had lower values of MLR preoperatively (and in

whom the average survival was 45 months, p=0.001), those in whom the preoperative value of NLR was high reported a survival of 15 months, significantly lower compared to those with low NLR - 44.2 months, p=0.001, those with high PLR reported an average survival of 17 months, significantly longer compared to those with preoperative low PLR (43.6 months, p=0.0001) while patients with high IIS had a median survival of only 15 months, significantly lower compared to those with low IIS – in which the median survival was of 44 months, p=0.001. These differences were also demonstrated when calculating the disease-free period. Thus, patients with CA 125, MLR; High NLR, PLR, and SII, respectively, benefited from a median disease-free survival of 11 months, 12 months, 13 months, 14 months, and 12 months, respectively, while cases with lower values of these parameters benefited from significantly disease-free survival higher – of 28 months, 33 months, 30 months, 28 months and 31 months respectively (p=0.003, p=0.002, p=0.001, p=0.01, p=0.006 respectively).

Conclusions

The detection of increased preoperative values of inflammatory markers is most often correlated with a large extension of the disease and, as a result, should represent a criterion for excluding patients from surgery as a first therapeutic alternative.

The correlation between nutritional and pro-inflammatory status in patients diagnosed with advanced stage ovarian cancer

Introduction and research hypothesis

Starting from the above observations that the inflammatory status of patients influences in a statistically significant manner the long-term evolution of patients with advanced ovarian neoplasms due to the fact that high inflammatory status is correlated with the synthesis of large amounts of cytokines such as interleukin 1 and respectively of interleukin 6 as well as tumor growth factors, we tried to see what other correlations can be established between various inflammatory parameters, nutritional status and the short term and respectively long-term evolution of patients diagnosed with advanced stage ovarian cancer. Thus, this time we focused our attention on a possible correlation between C reactive protein (C reactive protein - CRP), an acute phase protein whose synthesis is modulated by interleukins 1 and 6, and albumin, which is perhaps the most appropriate indicator of the patient's nutritional status. Other prognostic scores that take into account both inflammatory and nutritional status are the Glasgow score, the modified Glasgow score and the nutritional prognostic index, respectively. As a result, this time the research hypothesis refers to the existence of a correlation between the increased values of the CRP/albumin ratio, Glasgow score, modified Glasgow score and respectively low values of the nutritional prognosis index and the long-term evolution of advanced stage ovarian cancer patients.

Material and methods

The study group included the 57 patients subjected to primary cytoreduction, the group being made up after carefully analyzing the history of each patient, with the aim of not including possible cases with a recent history of other acute events that could cause the existence an elevated serum level of CRP. In all cases, we analyzed the preoperative level of CRP and serum albumin respectively, measured 24-48 hours before the surgical intervention. Later we determined the cut-off value of the CRP/albumin ratio, the target event being the achievement of complete cytoreduction. Thus, the threshold value of this ratio was set at 0.05, with a sensitivity of 0.8, 1-specificity of 0.75 and respectively with an area under the curve of 0.784. The Glasgow prognostic score was also calculated according to the preoperative values of CRP and albumin respectively as follows: CRP less than or equal to 10 mg/l, albumin greater than or equal to 35 g/l – 0 points, CRP less than 10 mg /l, albumin below 35 g/l 1 point, CRP above 10 mg/l and albumin above 35 g/l – 1 point and CRP above 10 mg/l and albumin below

35 g/l - 2 points. In the case of the modified Glasgow score, patients in the second category were also rated with 0 points instead of 1 point. The nutritional prognosis index was calculated as follows: albumin (g/l)+5*total number of lymphocytes*10^9/l and received 0 points if its value was above 48 and 1 point respectively if this value was below 48.

Results

Taking 0.05 as the cut-off value for the CRP/albumin ratio, the 57 patients were divided into two groups: those with a CRP/albumin ratio value below 0.05 – 9 cases and respectively those with a value of this ratio above 0 .05 – 48 cases. Overall, for the entire studied group, the average value of the CRP/albumin ratio was 0.41 (ranging between 0.02-1.56), the average value of the serum albumin level was 3.2 g/dl (between 1.6 and 4.4 g/dl) and the mean value of CRP was 11 g/dl (between 2 and 11 g/dl). When analyzing the correlations between the extent of resections and the preoperative values of the CRP/albumin ratio, we noticed that the extension of the disease in the upper abdomen and the need to perform resections at this level was not correlated with a higher level of the CRP/albumin ratio, a sign that the invasion in the upper abdomen should not be considered as a sign of a more aggressive tumor biology, but only of a neoplastic process that presented a longer evolution over time.

Regarding data on histopathological types and subtypes, we observed that patients in whom nonserous histology was confirmed had significantly higher serum levels of CRP/albumin ratio. As for the correlation between this parameter and the degree of differentiation, although the poorly differentiated subtypes had an increased value of the preoperative CRP/albumin ratio more frequently, this fact did not have statistical significance. When analyzing the possible correlation between the maximum size of the tumor, the value of the CRP/albumin ratio and respectively the status of the lymph nodes and this ratio, the existence of a statistically significant correlation did not emerge.

As for the immediate postoperative complications, they were recorded entirely among patients with increased values of the CRP/albumin ratio, this fact having marginal statistical significance (p=0.0495).

When analysing the long term outcomes, we observed that patients with low preoperative values of the CRP/albumin ratio had a significantly higher mean overall survival compared to those in whom this ratio was below 0.05 (6 months versus 9 months, p=0.01). Interestingly, however, when we analyzed the possible correlation between the CRP/albumin ratio and disease-free period, although patients with a low preoperative ratio had a longer

disease-free period, this fact was marginally statistically significant (14 months versus 6 months, p =0.05).

We further analysed the possible correlation between nutritional and inflammatory status accordingly to Glasgow score, modified Glasgow score and nutritional prognosis index respectively. Thus, according to Glasgow score, 19 patients had a score of 0, 21 patients had a score of 1 and the remaining 17 cases had a score of 2. According to the modified Glasgow score, 32 patients had a score of 0, 8 had a score of 1 and the remaining 17 had had a score of 2 and according to the nutritional prognosis index (PNI) there were 39 patients with PNI < 48 who received 1 point and respectively 18 patients with PNI > 48 who received 0 points. The analysis of various preoperative, intraoperative and postoperative parameters by prognostic groups according to the three scores presented above are shown in the tables below.

Table 4: the distribution of different perioperative parameters based on the Glasgow scale.

Parameter/number of cases	SG0	SG1	SG2	p
Age (years, mean value)	52,8	52,9	64,6	0,005
CA125 (U/dl)	547	2317	6223	0,0001
Ascites (ml, mean value)	1610	2009	3600	0,0002
NLR	2,1	3,6	7,3	0,0001
PLR	162	310	499	0,0001
MLR	0,24	0,45	1	0,0001
SII	634924	1383884	3532475	0,0004
Total serum protein (g/dl)	7,1	6,3	4,3	0,0001
Hb (g/dl)	12,9	12,2	10,2	0,03
CRP/Albumin	0,07	0,3	0,93	0,0001
Disease free survival (mean, months)	35	18	6	0,04
Overall survival (months)	42	27	12	0,044

As shown in the table above, there was a statistically significant correlation between the Glasgow score and the other bio-umoral parameters that translate the inflammatory and nutritional status of these patients, thus demonstrating again the close correlation between the two classes of variables (which define the systemic inflammatory response and respectively the nutritional status of the patient). We continued the analysis of the correlation between

inflammatory and nutritional status by following the distribution of various parameters according to the modified Glasgow score (SGM) (table 5). and respectively by the nutritional prognosis index (PNI) (table 6).

Table 5: the correlation between SGM and demographical, bio-umoral and survival rates of the study group

Parameter/no of cases	SGM			
	0	1	2	p
Age (years, mean)	51	58	64	0,02
CA125 (U/dl)	1014	3324	6223	0,0001
Ascites (ml, mean value)	1618	2625	3600	0,0001
NLR	2,6	4,13	7,36	0,001
MLR	0,29	0,61	1	0,001
SII	808231	1782716	3532475	0,001
CRP/albumin	0,14	0,41	0,94	0,02
Total serum protein (g/dl)	6,68	6,93	4,39	0,01
Hb (g/dl)	12,75	11,79	10,29	0,08
Disease free survival (mean, months)	31	13	6	0,001
Overall survival (mean, months)	50	19	12	0,001

Similarly to GS, we obtained statistically significant correlations regarding the SGM value and various other parameters quantifying the nutritional and inflammatory status respectively. Thus, as shown in the table above, patients with higher SGM had significantly higher levels of CA125, NLR, MLR, SII and CRP/albumin compared to those with lower SGM. Interestingly, this time, although the hemoglobin level was higher in those with a score of 0, compared to those with a score of 1 and respectively with a score of 2, the difference was not statistically significant. Meanwhile, patients with a score of 0 (those who had a good nutritional and immune status) tended to be younger (although the age difference was not statistically significant) and had fewer associated comorbidities.

When analyzing the impact of PNI on the perioperative evolution, we noticed that although there was no statistically significant correlation between PNI and more advanced

FIGO stages (p=1), it seems that patients in the first group presented less extensive lesions compared to those in the second group (translated by a lower serum level of CA125, a lower volume of ascites measured intraoperatively and significantly lower peritoneal carcinomatosis index). Interestingly, although this time there was no significant correlation between the PNI value and the histopathological subtype, it seems that well-differentiated tumors had more frequently a higher PNI, a correlation that was also established in regard to the presence of lymphatic metastases. This once again proves the importance of lymphocytes in lymphatic dissemination, patients with higher number of lymphocytes automatically having a higher PNI value and a significantly lower risk of lymphatic metastases.

Similarly to the data obtained when analyzing the other nutrition scores, patients with higher PNI had a significantly more resounding systemic inflammatory response, this being translated by significantly higher values of NLR, PLR, MLR and SII and respectively a poorer nutritional status defined by a significantly lower total protein serum level (7.19 g/dl vs. 5.49 g/dl, p=0.018). Surprisingly, we also did not obtain a statistically significant correlation between the serum hemoglobin level and PNI, although we would have expected that malnourished patients with a higher serum inflammation level would have a lower preoperative hemoglobin level compared to the rest of the group. However, this can be explained by the existence of multiple other mechanisms which come to explain the existence of anemia in neoplastic diseases. As for the need for extensive resections in the upper abdomen, they were comparable between the two groups thus demonstrating once again that tumors with extension in the upper abdomen do not present a more aggressive biology (they do not significantly influence the immune status of the host and do not induce immunosuppression).

When analyzing the immediate postoperative evolution, as expected, patients with poorer nutritional status and respectively with poorer protective status (caused by a lower number of circulating lymphocytes) developed postoperative complications much more frequently; moreover, all cases of death in the first 30 postoperative days were registered among patients with low PNI (respectively with PNI score=1).

Conclusions

Nutritional and inflammatory status are found in a close interrelationship in patients diagnosed with advanced stage ovarian cancers, providing valuable information regarding the long-term prognosis of these cases.

The correlation between procoagulant and proinflammatory status in patients diagnosed with advanced stage ovarian cancer

Introduction (research hypothesis)

As we demonstrated in the previous subsections, it seems that there is an extremely important correlation between the procoagulant status of patients diagnosed with advanced stage ovarian cancers and their evolution, both perioperative complications and survival rates being significantly lower in the cases presenting a higher number of circulating platelets during the preoperative studies. Later, when analyzing the impact of the pro-inflammatory status on the evolution of the study group, we demonstrated again that the existence of an accentuated pro-inflammatory status (demonstrated by the existence of increased values of NLR, MLR, SII, CRP) are invariably associated with an unfavorable long-term prognosis . Once these aspects have been demonstrated, in this subchapter we have investigated the possible correlations between the procoagulant and the proinflammatory status, as well as the way in which the two, cumulatively, could be considered as medium- and long-term prognostic factors in the case of advanced ovarian neoplasias.

Material and methods

And this time the studied group included the 57 patients undergoing primary cytoreduction in the Cantacuzino Clinic hospital, in all of them complete cytoreduction was attempted, surgery being the first therapeutic gesture. As in the previous studies, we took into account the data obtained from the patients' hemograms, all of which were collected 24 hours before the surgical intervention. And this time - we made sure that none of the patients included in the study had any history that could influence in a significant way the preoperative number of leukocytes and platelets - such as hematological diseases, splenectomy, prolonged administration of corticosteroids or septic conditions important in recent history. Later we investigated whether a correlation can be established between the proinflammatory and procoagulant status and the immediate and long-term prognosis of these patients. Thus, the cut-off value for the PLR ratio was set at 350, with a sensitivity of 0.9, 1-specificity of 0.78 and area under the curve of 0.78, respectively).

Results

Using the cut-off value of 350 for PLR, we divided the group of 57 patients into two subgroups: those with PLR<350 (37 cases) and respectively those with PLR>350 (20 cases). Later we analyzed the clinico-biological data of the patients from the two groups (table 6).

Table 6: the correlation ship between preoperative levels of PLR and clinical, biological and anatomopathological data of patients submitted to primary cytoreduction.

	PLR<350	PLR>350	P
Number of cases	37	20	
Age (years, mean)	53	62	P=0,015
FIGO stage: - IIIC - IV	 36 1	 17 3	 P=0,119
Histopathological stage - Serous - Endometroid - Mucinous - Clear cell carcinoma - Other histologies	 26 3 2 5 1	 13 1 1 1 4	P=0,539
Differentiation degree - I - II - III	 4 12 21	 2 3 15	P=0,449
Type of cytoreduction: - R0 - R1	 36 1	 9 13	 P<0,000001
Complicații postoperatorii: - Da - Nu	 4 33	 10 10	 P=0,0026
Associated comorbidities: - Yes - No	 14 23	 8 12	P=0,9
Mean hospital in stay (days)	10,3	10,5	P=0,9
CA125 (U/ml, mean)	334	2518	P<0,001

Platelets (*1000/microL, mean)	312575	469466	P<0,001
Lymphocytes(/microL, mean)	1781	897	P<0,001
Hemoglobin (g/dl)	12,39	10,34	P=0,004

As can be observed from Table 6, patients with lower PLR values were significantly younger and had a significantly lower rate of postoperative complications. Even so, however, the duration of hospitalization was similar between the two subcategories of the studied group. When analyzing the correlation between PLR and other biohumoral parameters, we noticed that patients with lower values of PLR had significantly lower values of CA125, circulating platelets, lymphocytes and hemoglobin, respectively.

Meanwhile, when calculating the long-term survival intervals, we observed that patients with higher PLR levels had a significantly worse evolution compared to those with PLR below 350. Thus, patients with higher PLR had a disease-free survival of only 7.7 months (95% confidence interval [CI] 6.5-10.4 months) while those with lower PLR had a disease-free survival of 22.5 months, 95% confidence interval [CI] 20, 1-29.3 months). The difference remained statistically significant in the overall survival analysis as well, being of 38.89 months (95% confidence interval [CI] 31-43 months) for patients with lower PLR and 13.7 months respectively for those with higher PLR levels - confidence interval [CI] 11.2-16.4 months) (p=0.01).

Conclusions

Based on the information presented above, we can conclude that there is an important correlation between the immune system, inflammatory and procoagulant status in the case of the investigated group; as a result, we can conclude that patients in whom there is an excessive inflammatory response in association with a significant procoagulant status have all the prerequisites to have a difficult evolution both short and long term evolution.

The correlation between leukocyte formula variables and the risk of lymphatic metastases

Introduction and research hypothesis

In previous chapters we have demonstrated that not all elements of the leukocyte formula seem to act synergistically in creating an unfavorable prognosis; thus, while monocytes and neutrophils appear to have a strong carcinogenic action by releasing various tumor growth factors and cytokines into the circulation, lymphocytes appear to play a central role in supporting the immune response and preventing tumor dissemination. Starting from these observations, we tried to find out in what proportion the various elements of the leukocyte formula are able to influence the medium and long-term evolution of patients with ovarian neoplasms. Once these elements were identified, we analyzed the pattern through which they lead to the appearance of metastatic disease.

Material and methods

This time we calculated the cut-off values for the MLR and NLR ratio, respectively, to have a predictive value on survival, also by means of ROC curves. Later, based on the cut-off values obtained by this method, we divided the study group into two subgroups and studied the various patterns of metastasis. So it appears that the parameter with the highest statistical power was the MLR (with an area under the curve of 0.783, sensitivity of 0.76, 1-sensitivity of 0.43, cut off value set at 0.32) , followed by NLR (with an area under the curve of 0.78, sensitivity of 0.8, 1-specificity = 0.438, cut-off value being 3.39).

Results

Based on the number of the preoperative MLR value, using the cut-off value of 0.32, the initial group of patients was divided into two subgroups, the one with the MLR value below the cut-off value - which included 25 patients and those with higher values respectively MLR - which included 32 patients. The clinico-demographic characteristics of the two groups are presented in the table below.

Table 7: the correlation between the preoperative MLR values and the perioperative and postoperative outcomes

Parameter	MLR<0,32 (n=25 cases)	MLR>0,32 (n=32 cases)	p
Age (mean, years)	51,52	60,22	0,2

CA125 (u/dl)	513	4751	0,003
Ascites (ml)	2456	3050	0,2
Albumin (g/dl)	6,8	2,9	0,001
Total serum proteins(g/dl)	6,9	5,3	0,018
Hemoglobin (g/dl)	12,5	11,3	0,8
Platelets	380784	435468	0,6
PLR	158,81	441,46	0,02
NLR	2,19	5,84	0,01
WBC (/microl)	9113	10868	0,858
CRP/albumin	0,11	0,66	0,022
Associated comorbidities: - Yes - No	 9 16	 13 19	 0,29
Postoperative complications: - Yes - No	 3 22	 13 19	0,02
Lymphatic metastases: - Yes - No	 6 19	 24 8	0,0002
PCI : - <10 - >10	 13 12	 1 31	0,0001
Type of resection: - Complete - Incomplete	 24 1	 22 10	0,01
Upper abdominal resections: - Da - Nu	 19 6	 21 11	0,56
Histopathological subtype: - Serous - Nonserous	 17 8	 22 10	1
Differentiation degree: - G1, G2 - G3	 14 11	 13 19	0,29
Disease free survival (mean, months)	20	9	0,01

Overall survival (mean, months)	54	18	0,001

And this time we observed a statistically significant difference between the serum level of CA 125, PLR, NLR and the PCR/albumin ratio and that of the preoperative MLR; interestingly, however, the volume of ascites determined preoperatively as well as the preoperative leukocyte level were similar between the two groups while the number of postoperative complications was significantly higher in the second group. At the time of the analysis of the intraoperative data, we noticed that although the resections in the upper abdomen were similar between the two groups, the index of peritoneal carcinomatosis as well as the rate of incomplete cytoreduction was much higher among patients with a higher MLR ratio compared to the cut value off. As for the pathological anatomy data, a significantly higher number of lymph node metastases was identified among patients with higher preoperative MLR levels (so probably with a poorer immune status, translated by a low preoperative lymphocyte count) . Later, we carried out in a similar manner the analysis of the impact of NLR on the status of the lymph nodes as well as their correlation with the rest of the clinical-biological data, the results being similar to those obtained with the MLR analysis.

Conclusions

Although the nodal status is often difficult to assess during preoperative investigations the extent of lymph node dissection being a highly controversial topic, the above data suggest that a careful analysis of preoperative bio-umoral data may draw attention to cases in which there is a high possibility that the nodes will be invaded. Once identified, these cases are expected to benefit most from extensive lymph node dissection.

The correlation between preoperative circulating platelet count, peritoneal and lymphatic dissemination

Introduction

As we demonstrated in the previous sub-chapters, it seems that there is an extremely important correlation between the procoagulant status and the extent of the lesions in patients with advanced stage ovarian cancer, one of the main incriminated mechanisms being the peritoneal one. Thus, it seems that the existence of a large number of circulating platelets is correlated with the extravasation and dissemination of neoplastic cells, an important mechanism by which this phenomenon occurs being the epithelial-mesenchymal transition. Once this process is initiated, the epithelial cells will acquire increased mobility and will migrate into the general circulation, thus leading to distant metastases.

Material and methods

At this moment the status of the lymph nodes at the level of the mesentery was investigated, given the fact that the existence of metastases at this level often represents an unfavorable prognostic factor thus representing a cause of incomplete debulking. The studied group was composed of the 57 patients undergoing primary cytoreduction, in which we investigated the existence of possible correlations between the preoperative platelet count, the status of the lymph nodes, the invasion of the digestive serosa, the need to perform resections in the upper abdomen, the existence of hematogenous liver metastases and the type of cytoreduction (complete/incomplete). And this time we calculated the cut-off value of the platelets with maximum predictive value on the long-term survival interval also by means of the ROC curve. Thus, this time, we determined a cut-off value of 335,000/microl, with a sensitivity of 0.8, 1-specificity =0.344, AUC=0.756.

Results

Applying a cut-off value of 335,000, the initial group of 57 patients was divided into two groups: one with PLT below 335,000 which included 27 patients and respectively the group with PLT above 335,000/microl which included 30 patients; in the first group the mean value of platelets was 272830/microl while in the second group the mean value was 452939/microl. Later, we analysed the presence of digestive serous invasion, mesenteric adenopathies, hepatic/splenic metastases occurring via hematogenous route and the feasibility of maximal debulking in the two groups, the results being presented in the following table.

Table 8: the correlation between the preoperative level of platelets and the presence of digestive invasion, mesenteric adenopathies, hematogenous metastases (hepatic or splenic) and, respectively, the type of cytoreduction.

	PLT<335.000/microl (n=27 cases)	PLT>335.000/microl (n=30 cases)	p
Mean number of PLT	272830	452939	0,02
Digestive invasion: - Yes - No	 9 18	 25 5	0,0002
Mesenteric lymph nodes - Yes - No	 2 25	 12 18	0,005
Hematogenous metastases - Yes - Nuo	 1 26	 9 21	0,001
Type of cytoreduction: - R0 - R1	 26 1	 20 10	0,006

As can be seen from the table above, we obtained a statistically significant difference between cases with a low platelet count and respectively those with a higher platelet count regarding the invasion of the digestive serosa, the presence of mesenteric adenopathies and hematogenous metastases, respectively. Also, patients with a lower preoperative platelet count benefited more frequently from maximal debulking.

Conclusions

In conclusion, we can state that ovarian cancer dissemination is intensively modulated by the homeostatic balance, by the procoagulant status and respectively by the existence of a large number of preoperatively detected platelets. Moreover, it seems that there are numerous connections between the various patterns of metastasis of this neoplasia, thus it is not possible to speak of an exclusively lymphatic, peritoneal or hematogenous route of metastasis. As demonstrated above, these pathways are often intertwined, a positive feedback between these elements being expected.

Prognostic factors at the time of secondary cytoreduction

Introduction and research hypothesis

Once the evolution of the group of patients subjected to primary cytoreduction was analysed, we noticed that only the initial FIGO stage or the type of cytoreduction seem to decisively influence the long-term evolution of these patients, but, perhaps more importantly, their evolution seems to be predicted by various clinical-biological parameters that are easy to be analysed from the pre-operative period, thus offering the possibility to the clinician and especially to the oncological surgeon to perform a more accurate selection of patients in whom surgical treatment per primam can provide a maximum survival benefit. Thus, as it results from the studies presented above, it seems that an accentuated procoagulant and respectively proinflammatory status seem to significantly influence the long-term evolution of these cases. Once this aspect has been demonstrated, in this subchapter we have investigated the characteristics of the group of patients undergoing secondary cytoreduction and to what extent the prognostic factors identified at the time of primary cytoreduction seem to influence the long-term evolution at the time of secondary cytoreduction.

Material and methods

At the time of conducting this study, in the period 2014-2020, we identified 18 patients undergoing secondary cytoreduction. Clinico-biological data from the time of surgery for first recurrence were reviewed retrospectively, as were data on disease-free period and type of surgery at the moment of initial diagnosis. The postoperative complications were quantified according to the Dindo Clavien scale.

Results

At the time of secondary cytoreduction, the average length of hospital stay was of 7 days (between 4 and 21 days) while the early postoperative mortality was 0. Considering the small number of cases submitted to secondary cytoreduction, we considered it inappropriate to perform an analysis of various factors of prognostic on this sample, however, we compared the average values of various preoperative parameters from the moment of secondary cytoreduction with those obtained on the study group submitted to primary cytoreduction. The patients at the time of the secondary cytoreduction had a worse nutritional status compared to those at the moment of primary cytoreduction and more frequently had a lower level of hemoglobin, these being considered as side effects of the adjuvant chemotherapy. Also, cases

undergoing secondary cytoreduction had a significantly lower number of platelets compared to those undergoing primary cytoreduction, this fact being explained by the impact that chemotherapy has on the myeloproliferative capacity as well as by the probable change of certain characteristics of malignant cells. A similar effect was observed on leukocytes in general but also on each individual line. Consequently, at the time of the secondary cytoreduction the absolute number of leukocytes, platelets and the hemoglobin value were much lower compared to those recorded at the time of the primary cytoreduction instead their ratio – PLR, MLR, NLR and respectively SII had comparative values with those reported at the time of primary cytoreduction as both parameters decreased.

On the other hand, when analysing the average natremia at the time of recurrence, it was lower (statistical significance being one at the limit - p=0.05) compared to that determined at the time of primary cytoreduction, the lower value of sodium at the time of recurrence being also given by the long history of chemotherapy as well as by the state of dehydration and electrolyte losses that can be induced sometime by prolonged chemotherapy administration. Considering these aspects, we tried to analyse these parameters before chemotherapy in order to eliminate its effect on the number of platelets, leukocytes and erythrocytes. Thus, we analysed the values of PLR, MLR and respectively NLR after primary cytoreduction in the 18 patients who later reached secondary cytoreduction and found that the patients in whom complete debulking was feasible at the time of secondary cytoreduction had a significantly greater decrease of these parameters after primary cytoreduction. Thus, the average values of PLR, MLR and respectively NLR after primary cytoreduction among patients in whom maximal debulking was feasible at the time of secondary cytoreduction was 124, 0.2 and 2.3, respectively, significantly lower compared to those in in which complete secondary cytoreduction was not feasible (and in which the mean values of the above parameters were 283, 0.4 and 3.8, respectively, p=0.002, p=0.003, p=0.007). As for the long-term evolution, this time it was influenced by the initial stage of the disease as well as the type of cytoreduction practiced both at the time of primary and secondary cytoreduction. Thus, patients whose initial stage was FIGO IIIB had an average survival of 30 months, while those who came from FIGO stages IIIC or IV – in which the average survival was 13 months (p=0.001).

Conclusions

The long-term evolution of relapsed ovarian cancer patients seem to be influenced by the initial stage of the disease as well as by the type of cytoreduction practiced both at the time of

primary and secondary cytoreduction. Thus, patients whose initial stage was FIGO IIIB had an average survival of 30 months, while those who came from FIGO stages IIIC or IV – in which the average survival was 13 months (p=0.001).

Conclusions and personal contributions

In the present study I included a group of 57 patients diagnosed with advanced stage ovarian cancer, subjected to primary cytoreduction as the first therapeutic strategy and respectively 18 patients subjected to secondary cytoreduction. As expected, both at the time of primary cytoreduction and at the time of secondary cytoreduction the long-term prognosis was decisively influenced by the initial stage of the disease and completeness of cytoreduction. However, even among patients who came from similar stages and in whom maximal debulking (defined by the absence of macroscopically visible residual tumor volume) was possible there were significant differences in distant evolution. This aspect enabled me to consider that there are other factors that decisively influence the prognosis of these patients. As a result, the impact of the procoagulant status (defined by the number of platelets and their average volume), of the inflammatory status (defined by the total number of leukocytes as well as by MLR, PLR, NLR, SII) as well as of the nutritional status (defined by the concentration serum albumin and total serum proteins) were analysed. The results showed that patients with an increased procoagulant status, as well as those with an accentuated proinflammatory status and respectively with a deficient nutritional status have a significantly worse prognosis compared to those with a correct fluid-coagulant balance, with a of good nutrition and respectively with a lower inflammatory status. Interestingly, however, we observed that the existence of a procoagulant status is correlated in the first phase with a massive peritoneal contamination and respectively with the existence of a higher index of peritoneal carcinomatosis, while an accentuated proinflammatory status is correlated in the first phase with the existence of tumors with a biological more aggressive translated by a lower degree of differentiation and which have a greater capacity to metastasize via the lymphatic route. As expected, cases with an intense inflammatory syndrome also showed significantly lower preoperative hemoglobin values as well as more frequent serious postoperative complications (Chapters 9-14).

However, in the penultimate chapter I demonstrated that there is an extremely interesting correlation between the three ways of metastasis - lymphatic, peritoneal and hematogenous respectively. Thus, it appears that an increased platelet count is initially correlated with greater peritoneal contamination; this contamination often occurs at the level of the digestive serosa, the tumor cells reaching this level behaving from a given moment in a manner similar to that observed in the case of colonic neoplasias. Thus, they will be taken over by the lymphatic capillaries at the level of the digestive tube serosa and will settle at the level of the lymphatic groups at the mesocolonic and respectively mesentery route. After reaching this level, the tumor cells will be taken over by the venous circulation and will reach the level of the portal vein, and further, through it, to the level of the liver parenchyma. Thus, it seems that peritoneal

contamination can be responsible form a certain moment for the apparition of unresectable lymph node metastases and even for the apparition of parenchymatous liver metastases. This observation is extremely useful in understanding the complex mechanism of dissemination through various patterns of this neoplasia (chapter 15).

Another interesting aspect that must be emphasized is the one related to the influence of these prognostic factors at the time of secondary cytoreduction. Thus, we demonstrated that at the time of recurrence there are numerous factors that influence all these parameters such as the extension of the neoplastic disease, the association of splenectomy at the time of initial cytoreduction, the prolonged history of chemotherapy, all these aspects making the parameters considered as prognostic factors at the time of primary cytoreduction to lose their predictive power at the time of secondary cytoreduction. However, we demonstrated that the value of these parameters, which are recorded after primary cytoreduction but before the administration of systemic treatments that could lead to the inhibition of the synthesis of various components, has an important value on long-term prognosis (chapter 16).

Based on these aspects, I consider that I have reached the objectives of the doctoral research, those of identifying new potential prognostic factors both at the time of initial diagnosis and at the time of diagnosis of the recurrence of ovarian neoplasms. Moreover, I demonstrated that an important number of these factors which are determinable in any laboratory, and which originate from the analysis of the routine constants monitored before any surgical intervention – the blood count and serum biochemistry respectively. Thus, the identification of these prognostic factors is feasible and advantageous from a technical-economic point of view, thus not presupposing the generation of additional costs and respectively not involving complicated imaging methods. Last but not least, I think that all this information deserves to be stored in various software that will then generate prediction models. Thus, we could hope that these results could constitute the base for the application of the concept of artificial intelligence in this field as well: once stored in large databases, the preoperative data of patients diagnosed with ovarian neoplasia will be able to be analyzed together with the data provided by the imaging and thus create the robot portrait of the patient with ovarian neoplasm. These data will be able to serve in the future to identify patients predisposed to the appearance of this neoplasia or at least to diagnose it in its early stages. Once the desirability of early diagnosis in ovarian neoplasm is achieved, we can hope that long-term survival will improve considerably.

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