

Changes of Peripheral Lymphocyte Populations in Patients with Advanced/Recurrent Ovarian Cancer Undergoing Splenectomy During Cytoreductive Surgery

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Research

Keywords: Ovarian Neoplasms, Lymphocyte, Subpopulation, Flow cytometry, Splenectomy, Cytoreductive surgery

Posted Date: June 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-33275/v1>

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Abstract

Background: To investigate the change of peripheral lymphocyte subsets after splenectomy during cytoreductive surgery for advanced or recurrent ovarian cancers

Methods: We enrolled 83 patients with advanced or recurrent ovarian cancer who underwent cytoreductive surgery between 09/2016 and 01/2019. Twenty patients who also underwent splenectomy were assigned to the splenectomy cohort and the rest to the non-splenectomy cohort. Flow cytometry was used to measure the peripheral lymphocyte subsets consisting of T cells, regulatory T cells, natural killer cells, B cells, and activation antigens before and after surgery.

Results: There was not any difference in the number and distribution of peripheral lymphocyte subsets between the two cohorts before surgery. We observed elevated levels of T cells (CD3⁺, CD3⁺CD8⁺) in the splenectomy cohort compared to those in the non-splenectomy cohort after surgery, which achieved statistical significance. The post-operative CD4/CD8 ratio was lower in the splenectomy cohort than in the non-splenectomy cohort ($P=0.048$). Regarding the changes of lymphocyte subsets after surgery, CD8⁺CD28⁺ T cells had a significant decreasing tendency ($P=0.011$) while CD3⁺/HLA-DR⁺ T cells were the opposite ($P=0.001$) in the splenectomy cohort. In the non-splenectomy cohort, the proportion of Tregs ($P=0.005$) and B cells ($P<0.001$) including CD3⁻/HLA-DR⁺ B cells ($P=0.007$) increased after surgery, and the absolute number of T cells and NK cells decreased to different extents ($P<0.001$). The post-operative percentage of CD8⁺CD28⁺ T cells was less than the pre-operative one ($P=0.022$), which was similar to the splenectomy cohort.

Conclusions: The changes of peripheral lymphocyte populations were different between patients with splenectomy and without splenectomy during cytoreductive surgery for advanced or recurrent ovarian cancers. T cells were increased and activated in splenectomy cohort, whereas, B cells were increased and activated in non-splenectomy.

Background

Ovarian cancer is the most lethal gynecologic malignancy^[1]. In China, more than 50,000 new cases and nearly half of the deaths have occurred in 2016, representing the second-highest cause of gynecologic cancer-related death^[2]. It is well accepted that optimal cytoreduction improves survival in ovarian cancer patients^[3, 4]. In some circumstances, removal of the spleen as part of upper abdominal surgery is required to achieve complete resection^[5, 6].

As the spleen is one of the most important peripheral immune organs, splenectomy has been suggested to have an impact on immunological function^[7]. Some studies found that people with splenectomy have a higher risk of developing overall cancer, as well as certain site-specific cancers^[8, 9]. However, the spleen's anti-tumor function is bidirectional, which means that it has a positive anti-tumor role in the early stage of tumor formation, and also a negative immunological impact on tumor progression^[10, 11].

Peripheral lymphocytes that directly reflect the patients' immune status might be influenced by a variety of factors, including surgery especially splenectomy. It is not clear whether splenectomy has a beneficial effect on immune function in advanced or recurrent ovarian cancer patients, beyond the benefit of reducing tumor.

In the present study, we aimed to investigate the effect of cytoreductive surgery with or without splenectomy on the immunological status of advanced and recurrent ovarian cancer patients. We compared the pre- and post-operative circulating lymphocyte distribution, and analyzed the changing trends of the two cohorts.

Materials And Methods

Patient and comparison groups

The study was approved by the ethics committee of Fudan University Shanghai Cancer Center. All patients signed informed content. Patients were included who underwent optimal cytoreductive surgery for advanced or recurrent epithelial ovarian cancer between September 2016 and January 2019. The staging was according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system (2014) ^[12]. Optimal cytoreductive surgery was defined as residual disease less than (or including) 1 cm after debulking surgery. Twenty patients received splenectomy during cytoreductive surgery for the tumor involvement of spleen. Other patients were grouped into the non- splenectomy group. Patients were excluded in this study if the FIGO stage was less than IIIC, non-epithelial ovarian cancer, presence of other tumors, presence of active infections or immune disease, or lacking complete data.

Clinicopathological features were retrospectively abstracted from the electronic medical records, including age at first diagnosis in our institution, FIGO stage, histological subtypes, pre-treatment serum CA 125, residual disease, and adjuvant chemotherapy. Recurrent diseases were classified into platinum-sensitive disease if the interval time was > 6 months from the completion of the last platinum-based chemotherapy to the date of disease recurrence, and platinum-resistance disease if the interval time was < 6 months.

Blood sample collection and flow cytometry

Written informed consent was acquired at admission. Peripheral blood samples were collected in heparinized tubes before treatment and after treatment. The average time interval from blood collection to surgery is three days while post-operative blood samples were also collected before discharge, usually one week after the operation. Blood cells were treated with red blood cell lysis buffer (Beyotime, China) and then labeled with fluorochrome-conjugated monoclonal antibodies and incubated at 4°C for 20 min. Detailed procedures of flow cytometry using BD Multitest 6-color TBNK reagent (BD Pharmingen, USA) have been described in a previous publication from our institution ^[13].

Statistical analysis

Statistical Package for Social Science (SPSS) (Version 20.0, SPSS, Inc., Chicago, IL, USA) was used to analyze all data, and GraphPad Prism (Version 6.0, GraphPad Software, Inc., La Jolla, CA, USA) was utilized to create the artwork. Mean \pm standard deviation (SD) was applied for continuous variables, while absolute numbers followed by percentage (%) for categorical data. Binomial data from two groups underwent chi-square analysis, and parametric data had Independent-Samples T-tests to seek any differences of characteristics. Lymphocyte subpopulations, non-normally distributed, are shown as median (quartile range, QR). The difference between the groups was determined using the Mann-Whitney U test. Changes in lymphocyte subsets in patients undergoing different surgeries were evaluated using the Wilcoxon rank test. All *P* values reported were two-tailed, and a *P*-value < 0.05 was regarded as statistically significant.

Results

Patient characteristics

During the period, a total of 108 patients received cytoreductive surgeries for advanced or recurrent ovarian cancer. Of them, 25 patients were excluded due to incomplete data. Among the rest 83 patients, 20 patients were grouped into the splenectomy cohort (splenectomy during cytoreductive surgery) due to splenic metastasis, and 63 patients were grouped into the non-splenectomy cohort (cytoreductive surgery without splenectomy). At the time of analysis, 16 patients in the splenectomy cohort and 40 patients in the non-splenectomy cohort were available for platinum response assessment. The study participant flow diagram is shown in Fig. 1.

Table 1 shows the demographic and clinicopathological characteristics of the 83 patients. Primary diseases accounted for 70% and 67.5% of the cases in the splenectomy group and non-splenectomy group. The age of the patients at the time of diagnosis and the level of CA125 before treatment was not statistically different between the two groups. Most of the patients have high-grade serous carcinomas (90% and 87.5%). Two patients in the splenectomy group received neoadjuvant chemotherapy before surgery, but no significant difference was noted ($P = 0.056$). All patients received optimal debulking with residual disease smaller than 1 cm, while more than 60% of the cases achieved complete resection (65% in the splenectomy group vs. 63.5% in non-splenectomy group, respectively). Clearly shown in Fig. 1, 56 patients were available for platinum response assessment. Of them, platinum-resistant recurrence accounted for 37.5% in the splenectomy group and 15.0% in the non-splenectomy group, respectively, which was not statistically significant.

Table 1
Demographic and clinicopathological information.

Variables	Splenectomy (n = 20)	Non-splenectomy (n = 63)	P-value
Age (years), mean (SD, 95% CI)	58 (9.7, 53–62)	54 (8.8, 52–56)	0.114*
Neoadjuvant chemotherapy (%)	2 (10%)	0	0.056 [#]
Histology			
High-grade serous carcinoma	18 (90.0%)	55 (87.3%)	0.246 [#]
Clear cell carcinoma	0	5 (7.9%)	
Endometrioid	1 (5.0%)	1 (1.6%)	
Carcinosarcoma	1 (5.0%)	0	
Low-grade serous carcinoma	0	2 (3.2%)	
Pre-treatment CA125 (U/mL), media (range)	718.9 (8.9–5000) ^a	539.0 (8.3–5000)	0.446 ^{**}
FIGO stage (%)			
IIIC	11 (55.0%)	33 (52.4%)	0.838 [#]
IV	3 (15.0%)	14 (22.2%)	
Recurrence	6 (30.0%)	16 (25.4%)	
Residual disease (%)			
0	13 (65.0%)	40 (63.5%)	0.903 [#]
≤1 cm (optimal)	7 (35.0%)	23 (36.5%)	
Platinum response (%) ^b			
Sensitive	10 (50.0%)	34 (54.0%)	0.093 [#]
Resistant	6 (30.0%)	6 (9.5%)	

Table 2

Distributions of peripheral lymphocyte subpopulations in two groups before and after surgery.

Peripheral lymphocyte subsets	Splenectomy group			Non-splenectomy group			P_1	P_2
	pre	post	P_3	pre	post	P_4		
Percentage (%)								
T cells (CD3 ⁺)	67.8 (17.3)	68.9 (16.0)	0.241	67.6 (14.48)	69.1 (12.9)	0.276	0.680	0.431
CD3 ⁺ CD8 ⁺	30.1 (12.2)	26.2 (16.5)	0.210	25.2 (12.50)	27.1 (14.5)	0.056	0.109	0.289
CD3 ⁺ CD4 ⁺	37.7 (7.1)	35.5 (9.2)	0.768	38.2 (12.8)	39.7 (12.8)	0.292	0.616	0.070
CD4/CD8	1.2 (0.8)	1.2 (0.8)	0.746	1.6 (0.9)	1.7 (1.3)	0.038	0.125	0.048
CD8 ⁺ CD28 ⁺	8.9 (4.1)	7.6 (3.9)	0.011	6.9 (4.3)	6.8 (3.4)	0.022	0.116	0.606
NK cells (CD3 ⁻ CD16 ⁺ 56 ⁺)	16.1 (12.9)	11.2 (13.9)	0.312	15.8 (14.0)	13.5 (13.3)	0.043	0.721	0.563
B cells (CD3 ⁻ CD19 ⁺)	10.5 (9.5)	11.4 (8.6)	0.922	11.3 (6.9)	13.9 (7.7)	< 0.001	0.880	0.195
Tregs (CD4 ⁺ CD25 ⁺ CD127 ^{low})	13.2 (3.3)	14.6 (4.1)	0.344	12.8 (4.0)	13.2 (4.2)	0.005	0.477	0.461
HLA-DR ⁺	22.5 (13.6)	26.2 (19.1)	0.011	22.8 (18.2)	26.3 (15.8)	0.003	0.812	0.864
CD3 ⁺ /HLA-DR ⁺	6.3 (17.4)	9.3 (19.4)	0.001	11.3 (13.9)	11.3 (14.6)	0.198	0.563	0.969
CD3 ⁻ /HLA-DR ⁺	10.3 (8.5)	13.7 (11.2)	0.177	11.6 (6.8)	13.6 (6.3)	0.010	0.570	0.843
CD3 ⁺ /HLA-DR ⁻	59.2 (17.3)	49.6 (22.6)	< 0.001	57.4 (17.2)	55.9 (18.6)	0.002	0.669	0.093
Absolute number (cell/ul)								
T cells (CD3 ⁺)	942 (522)	1079 (502)	0.522	1101 (466)	821 (543)	< 0.001	0.226	0.013
CD3 ⁺ CD8 ⁺	406 (290)	411 (261)	0.709	416 (251)	313 (237)	< 0.001	0.868	0.012
CD3 ⁺ CD4 ⁺	495 (273)	611 (263)	0.490	594 (416)	465 (311)	< 0.001	0.111	0.140

Peripheral lymphocyte subsets	Splenectomy group			Non-splenectomy group			P_1	P_2
	pre	post	P_3	pre	post	P_4		
NK cells (CD3 ⁻ CD16 ⁺ 56 ⁺)	201 (47)	161 (276)	0.595	255 (236)	162 (173)	< 0.001	0.250	0.510
B cells (CD3 ⁻ CD19 ⁺)	152 (194)	149 (158)	0.241	164 (133)	160 (147)	0.380	0.572	0.570

^a Numbers were presented as median (quartile rang).

^b Pre = before surgery; Post = after surgery.

^c P values with statistical significance were denoted. P_1 = Splenectomy vs. Control before surgery; P_2 = Splenectomy vs. Control after surgery; P_3 = Preoperative vs. Postoperative in the splenectomy cohort; P_4 = Preoperative vs. Postoperative in the control cohort.

Change trends before and after surgery: Splenectomy vs. Non-splenectomy group

As shown in Fig. 3, in the splenectomy cohort, the median percentage of CD8⁺CD28⁺ T cells levels significantly decreased after the operation (pre-operative vs. post-operative: 8.9 vs. 7.6, $P = 0.011$), while the median percentage of activation antigens significantly increased (HLA-DR⁺, pre-operative vs. post-operative: 22.5 vs. 26.2, $P = 0.011$), which was mainly reflected in the increase of the percentage of activated CD3⁺/HLA-DR⁺ T cells (preoperative vs. post-operative: 6.3 vs. 9.3, $P = 0.001$), with the decline of the percentage of CD3⁺/HLA-DR⁻ (pre-operative vs. post-operative: 59.2 vs. 49.6, $P < 0.001$). Other variables remained unchanged before and after the operation.

Correspondingly, in addition to the percentage of T cells, almost all other variables changed significantly after debulking in the non-splenectomy cohort. The absolute numbers (presented in Fig. 4) of post-operative T cells (CD3⁺, CD3⁺CD8⁺, CD3⁺CD4⁺) were lower than preoperative ones ($P < 0.001$). The absolute number and percentage of post-operative NK cells (CD3⁻CD16⁺56⁺) also declined (absolute number: 255 vs. 162, $P < 0.001$; percentage: 15.8 vs. 13.5, $P = 0.043$). There were no significant differences in the absolute number of B cells (CD3⁻CD19⁺), but the percentage was higher than that before surgery ($P < 0.001$) which was mainly due to decreased absolute numbers of T cells and NK cells. The post-operative percentage of Tregs (CD4⁺CD25⁺CD127^{low}) was significantly greater than the pre-operative one ($P = 0.005$). The post-operative percentage of CD8⁺CD28⁺ T cells went down ($P = 0.022$) and the activation antigens (HLA⁻DR⁺) went up ($P = 0.003$).

In terms of absolute numbers of peripheral lymphocyte subpopulations, the changes of B cells were not significant in the two cohorts, while the NK cells decreased, in which the non-splenectomy cohort showed

significant changes ($P < 0.001$). The post-operative T cells' value in the splenectomy group went up ($P > 0.05$), while in the non-splenectomy group went down ($P < 0.01$). In terms of the percentage, elevated levels of B cells and Tregs as declining levels of NK cells were found in both groups. These changes in the non-splenectomy group were significant ($P < 0.05$), and more obvious than that of the splenectomy group. $CD8^+CD28^+$ T cells levels of both cohorts decreased significantly ($P < 0.05$), but only the CD4/CD8 ratio of non-splenectomy cohort increased ($P = 0.038$). After debulking, the proportion of lymphocytes with activated antigens (HLA-DR⁺) was greater than before in both groups. The changes were reflected in a significant increase in the percentage of activated T cells ($CD3^+/HLA-DR^+$) in the splenectomy cohort ($P = 0.001$), compared with activated B cells ($CD3^-/HLA-DR^+$) in the non-splenectomy cohort ($P = 0.007$).

In summary, the changes of peripheral lymphocyte subpopulations were not completely identical in patients with splenectomy and without splenectomy during debulking surgeries for advanced or recurrent ovarian cancers. $CD3^+$ T cells and $CD8^+$ T cells were significantly increased in patients with splenectomy, whereas, decreased in patients without splenectomy. Activation antigen (HLA-DR⁺) was increased in T cells from the patients with splenectomy and in B cells from the patients without splenectomy. In the non-splenectomy group, NK cells significantly decreased and Tregs significantly increased after surgery, which did not reach statistical significance in the splenectomy group. $CD8^+CD28^+$ T cells are decreased in both groups. And B cells were not significantly changed after surgery in both groups.

Discussion

It has been well accepted that patients with advanced tumors present an immunosuppressive status^[14]. The spleen, as the most important peripheral immune organ, plays a negative immunologic role in it^[7]. Surgery is the standard treatment of OC and may assist in restoring the immune response^[15]. A report suggested that the anti-tumor effect of the immunologic function of the spleen was restored when the negative factor resulting in immune suppression produced by tumor cells was eliminated by debulking surgery^[11]. Our results showed that the decrease of postoperative $CD8^+CD28^+$ T cells and NK cells in both groups indicated that the postoperative anti-tumor immunity of ovarian cancer patients was suppressed. Immune functional changes after surgery were less apparent in the ovarian cancer patients with splenectomy compared to those without splenectomy. The changes, such as the decrease in the absolute number of T cells and NK cells, the increase of the proportion of Tregs ($CD4^+CD25^+$) and B cells, did not occur in the splenectomy group. Correspondingly, T cells levels of $CD3^+$ and $CD3^+CD8^+$ in the non-splenectomy group were significantly lower than those in the splenectomy group. All these might suggest that the spleen played a part in postoperative immunosuppression in mediating the changes of peripheral blood lymphocytes. So, splenectomy could reduce the immune suppression of ovarian cancer patients caused by surgery.

Increased circulating Tregs have been reported in patients with different kinds of malignant tumors, including ovarian cancer^[13,16-18]. Studies have considered that Tregs mainly stores in the spleen^[19]. They might be set free by the spleen into circulation during post-operative immune rearrangement, which

could explain why CD4⁺ Tregs increased in patients with spleen preservation after surgery. CD4⁺ Tregs, one of the major Tregs subtypes reported to date, can inhibit the tumor-specific T cell-mediated immune response and contribute to tumor escape and poor survival [20, 21].

The role of B cells in ovarian cancer is difficult to determine. An experiment in the laboratory indicated that B cells migrated to the site of the tumor and acquire expression of immunosuppressive ligands and/or cytokines that contributed to the inhibition of anti-tumor immune response [22]. Another publication showed that B cells infiltrating high-grade serous ovarian cancer omental metastases supported the development of an antitumor response [23]. The antitumor effect of B cells in peripheral circulation is rarely studied. In our study, the reason for the increased B cells proportions may be the same as that for Tregs, since the spleen is the largest B cell producing organ. The spleen might play a role in regulating the number of B cells in peripheral blood and keeping stable when the depression of the immune response was evoked owing to the serious attack from surgical trauma. Therefore, the immune benefit is limited, because the increase of B cell proportion is based on the decrease of T and NK cell proportion. The absolute number did not increase.

In the splenectomy group, the changes in immune status were not so obvious as those in the non-splenectomy group. We noted that the proportion of activated antigens (HLA-DR⁺) increased within 1–2 weeks post-operatively. It was taken into account in T cells (CD3⁺/HLA-DR⁺) of splenectomy group and B cells (CD3⁻/HLA-DR⁺) of the non-splenectomy group. Studies on the role of HLA-DR⁺ in tumor immunity were rare and remain controversial. Our previous related study identified that an HLA-DR Tregs population in the peripheral blood was significantly increased in cervical cancer patients compared to precancer patients and healthy donors [24]. Activated T cells (CD3⁺/HLA-DR⁺) were found to increase during chemotherapy for non-Hodgkin's lymphoma patients, then reduce after chemotherapy, and the elevated level was associated with recurrence [25]. For ovarian cancer patients, the activated T cells have proved to be related to the size of residual tumors [26]. Under normal conditions, the majority of T cells are CD4⁺ T helper (Th) 1 and CD8⁺ T cytotoxic (Tc)1 cell. Th1/Tc1 immune responses, enhance HLA-DR expression in T cells in the inflamed sites, can lead to an increase in CD3⁺HLA-DR⁺ extracellular vesicle levels in the circulation [27]. Recent progress in cancer immunotherapy has disclosed that Th1/Tc1 responses are suppressed in cancer patients and they are restored by blockade of immune checkpoint receptor PD-1 or PD-L1 [28]. In our study, post-operative activated T cell elevation was unique to the splenectomy group. Regardless of whether this phenomenon was caused by post-operative inflammatory stimulation or decreased tumor load, it seemed to provide diverse ideas for postoperative immunotherapy. Kikuchi et.al found that the percentage and absolute number of CD3⁻/HLA-DR⁺ (B cells) in peripheral blood of patients with advanced ovarian carcinoma were significantly lower than values from patients with benign ovarian tumor [26]. In the present study, we found an increase in CD3⁻/HLA-DR⁺ cells post-operatively only in the spleen-preserving group. Whether the increased activation of B cells can promote tumor immunity remains to further study.

The study presents several limitations. Firstly, a one-time snapshot of lymphocyte values could not reflect the kinetics. Prolonged observation might lead to a different conclusion considering the time needed for the immune function reaches a different balance. We intend to collect consecutive blood samples for further study. Secondly, due to short follow-up time, evaluation of platinum resistance was only obtainable in some patients and the survival outcome was not analyzed. We will update the survival information and investigate the possible prognostic implication of lymphocyte variables. Thirdly, we only analyzed the lymphocyte subsets mentioned above, and additional tests were required for other subsets. Lastly, given the limited case number, the result might partly explain the insignificant differences between ovarian cancer patients with and without splenectomy.

Conclusion

The release of spleen function after surgery does not necessarily reflect to remove the immunosuppression but seems to intensify tumor immune escape within two weeks after surgery. Ovarian cancer debulking combined with splenectomy has a little negative effect on peripheral lymphocyte subpopulation distribution, but is beneficial to the balance of T cell subsets and improve the antitumor immune function. It is advantageous to the post-operative chemotherapy and immunotherapy as soon as possible after surgery.

Abbreviations

Tregs: regulatory T cells; NK: natural killer; FIGO: The International Federation of Gynecology and Obstetrics; CA: cancer antigen.

Declarations

Ethics approval and consent to participate:

The study was approved by the Fudan University Shanghai Cancer Center review board and the requirement for written informed consent was waived due to its retrospective design.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

Funding:

No funding.

Authors' contributions:

WC, SY, YW, XP, LX, BP, BS, and HY contributed to the conceptualization and design of the study. WC, SY, YW, and LX collected and analyzed patients' clinicopathological data. XP, BP, and BS performed and analyzed the flow cytometry. WC, SY, BS, and HY were major contributors in writing the manuscript. WC, SY, YW, XP, LX, BP, BS, and HY read and approved the final manuscript.

Acknowledgments:

Not applicable

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Figures

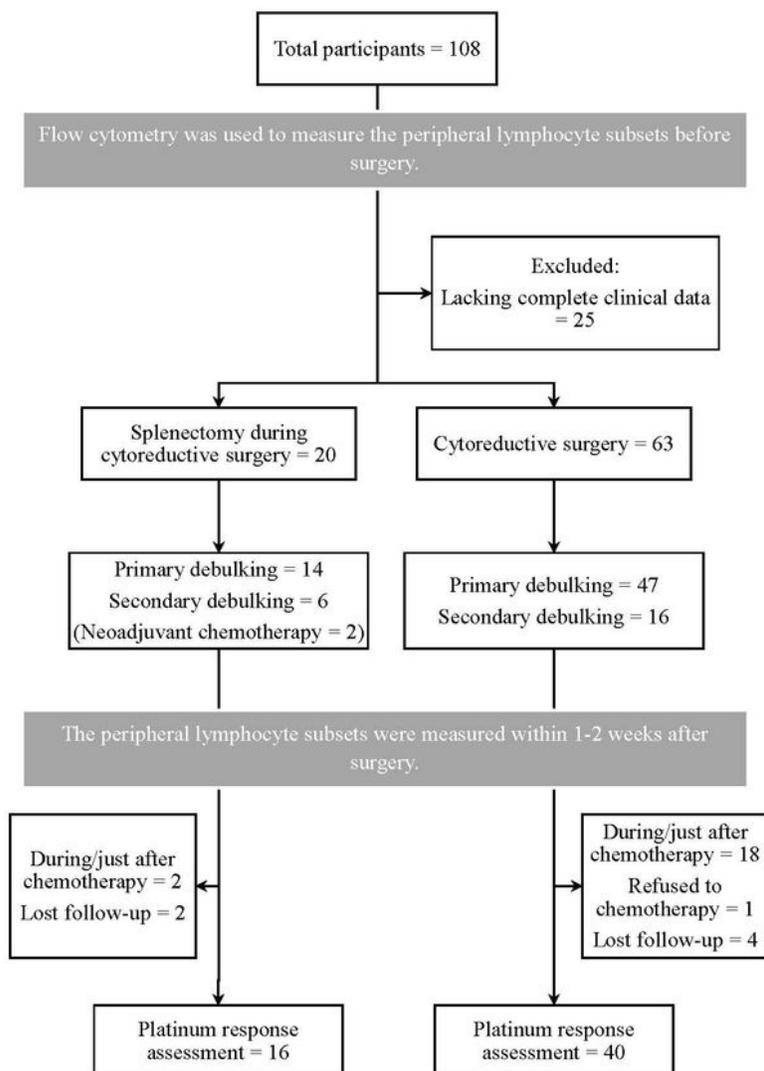


Figure 1

Schematic flowchart of the patients contained in the present study.

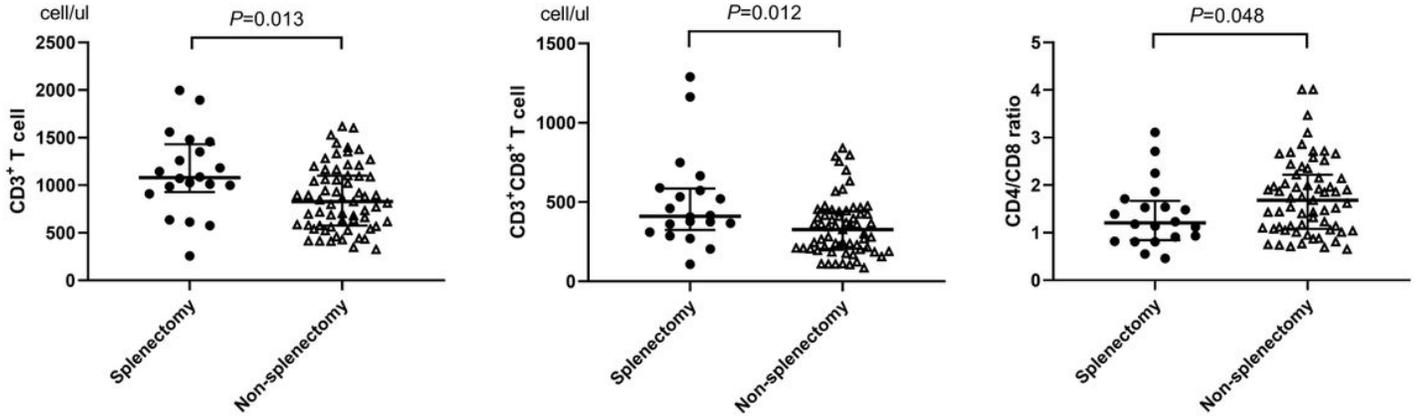


Figure 2

Comparisons with the statistical significance of circulating lymphocyte subsets after surgery (Splenectomy Vs. Non-splenectomy).

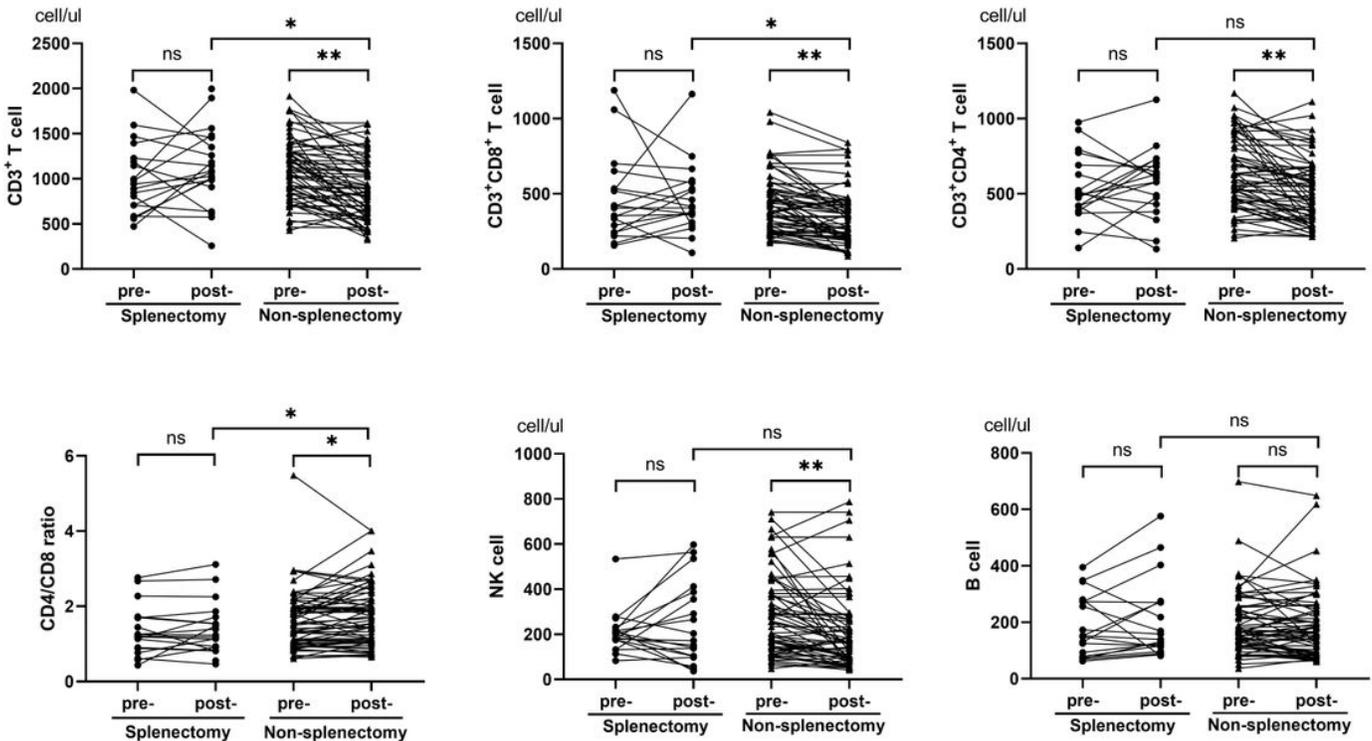


Figure 3

Changes in peripheral lymphocyte absolute numbers in the two cohorts after upfront surgery. Abbreviations: Pre- = before surgery; Post- = after surgery. ns=not significant; *, P < 0.05; ** P < 0.01.

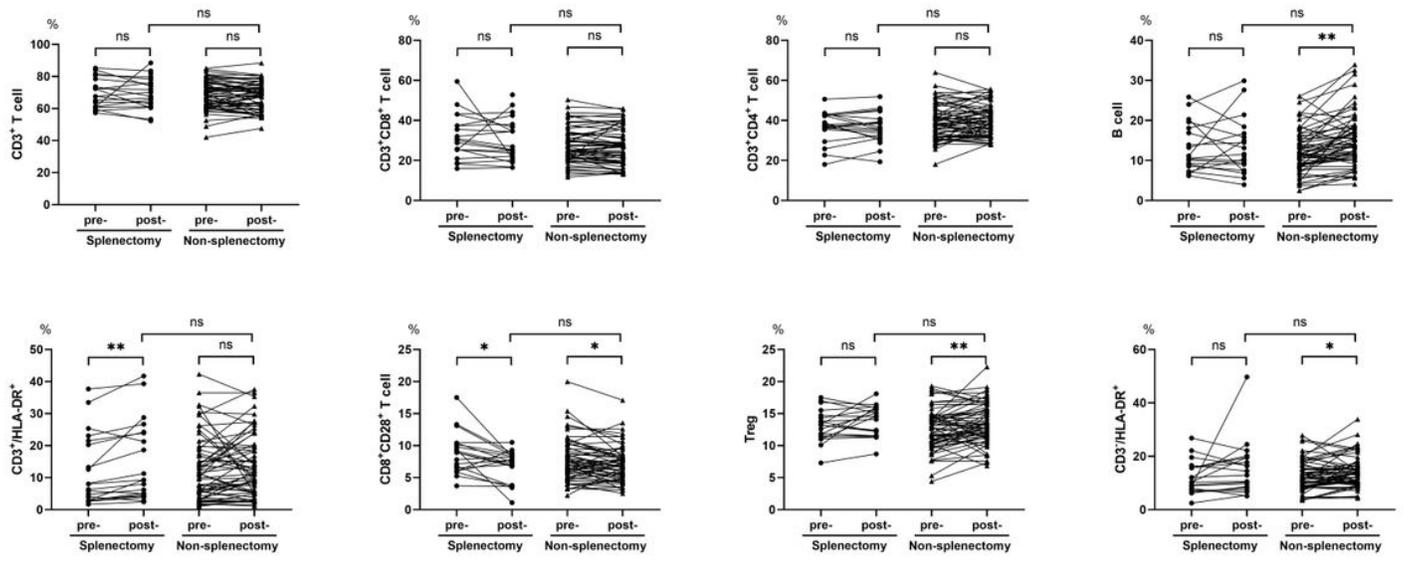


Figure 4

Changes in peripheral lymphocyte distribution in the two cohorts after upfront surgery. Abbreviations: Pre- = before surgery; Post- = after surgery. NS=not significant; *, P<0.05; ** P <0.01.