Dynamic Peripheral Blood Lymphocyte Subsets as Predictive Biomarkers for Langerhans Cell Histiocytosis in Children

Yuhua Feng (fyh87256630@csu.edu.cn)
Second Xiangya Hospital of Central South University
Leyuan Wang
Xiangya Hospital Central South University
Yan Yu
Xiangya Hospital Central South University
Liangchun Yang
Xiangya Hospital Central South University

Research Article

Keywords: Langerhans cell histiocytosis, Peripheral blood lymphocyte subsets, Prognosis

Posted Date: May 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1644798/v1

Abstract

Background: Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasm, immune system dysfunction may attribute to the cause of LCH. There is no standard prognosis evaluation system for LCH in children. We investigated the prognosis predictive value of peripheral lymphocyte subsets in childhood LCH patients.

Methods: A cohort of 79 childhood LCH patients was retrospectively studied. The prognosis predictive significance of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells, B cells and CD4/CD8 ratio were analyzed.

Results: After 6-week induction therapy, the percentage of $CD3^+$ T cells, $CD3^+CD4^+$ T cells and CD4/CD8 ratio were significantly increased, while $CD3^+CD8^+$ T cells and B cells were decreased. $CD3^+CD8^+$ T cells and B cells were closely related to *BRAF V600E* and *MAP2K1* mutation. $\Delta CD3^+$ T cells, $\Delta CD3^+CD4^+$ T cells, $\Delta CD3^+CD8^+$ T cells, ΔNK cells and ΔB cells were related to treatment efficacy. As for organ involvements, $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were related to liver involvement, ΔCD4/CD8 ratio was related to CNS involvement $\Delta CD3^+$ T cells and ΔB cells were related to *BRAF V600E* mutation, while $\Delta CD3^+CD4^+$ T cells was related to *MAP2K1* mutation. Furthermore, increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD4^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells, decreased ΔNK cells and increased ΔB cells were predictors for superior PFS, while increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells and decreased ΔNK cells were predictors for superior OS. In addition, multivariate Cox regression analysis showed that $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were independent prognostic factors for PFS.

Conclusions: The peripheral lymphocyte subsets including $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells and B cells might be promising predictive indicators for LCH in children.

Introduction

Langerhans cell histiocytosis (LCH) is a disorder characterized by abnormal proliferation of Langerhans cells. The cause of LCH remains unknown, immune system dysfunction may be involved. The manifestation of LCH varies widely from single-system to multi-system diseases[1]. Although the standard treatment regimen has improved the outcome of LCH in children, more than 50% patients develop relapse/refractory disease. Patients with high-risk organs involved present with poor prognosis. However, there is no standard prognostic score system for LCH in children.

Advances in genomic sequencing techniques lead to deeper understand of LCH[2]. Previous studies reported that *BRAF V600E* mutation indicated severer disease and resistance to induction therapy[3, 4]. Besides *BRAF V600E*, *MAP2K1* is also thought to play roles in LCH, *MAP2K1* mutation was related to risk organs involvement[5]. However, *BRAF V600E* and *MAP2K1* mutation are not enough to predict the prognosis of LCH.

Recent studies have shown that immune system is closely related to the development and prognosis of cancers[6]. Immune surveillance during treatment is important. Peripheral lymphocyte subsets can be used as a non-invasive and effective method for immune surveillance, previous reports also confirmed their values in predicting prognosis of lung cancer[7], breast cancer[8], laryngeal squamous cell cancer[9], etc.. However, there is few studies on peripheral lymphocyte subsets in LCH.

We evaluated the prognostic values of peripheral lymphocyte subsets including $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells and B cells in 79 childhood LCH patients.

Materials & Methods

Patient Selection

A cohort of 79 newly diagnosed childhood LCH patients were retrospectively studied. These patients were diagnosed from January 2014 to December 2021 at the Xiangya Hospital, Central South University. The inclusion criteria were as follows: a) pathological confirmed LCH; b) age ≤ 14 years. The exclusion criteria were the following: a) received treatment including radiotherapy, chemotherapy; b) autoimmune disease history; c) chronic inflammatory disease history; d) active infectious disease.

All the 79 LCH patients had complete follow-up and clinical data. These patients were followed-up from the day of diagnosis to April 2022, no patients lost to follow-up. The study was conducted in accordance with the Declaration of Helsinki of 1975, which was revised in 2008.

Data Collection

Clinical data, including gender, age, Histiocyte Society (HS) classification, organ involvement, *BRAF V600E* and *MAP2K1* status and treatment efficacy after 6-week induction therapy which conducted LCH-III protocol—vinblastine and prednisone[10], were obtained. Progression free survival (PFS) denotes the time length from diagnostic date to dates of death or disease progression. Overall survival (OS) is the time length between diagnostic date to the date of death due to any cause or to last follow-up date.

Flow cytometry

2ml peripheral blood from each LCH patients before and after 6-week induction therapy was collected in EDTA anticoagulant vacuum vascular. 20μl premixed monoclonal antibody and homotypic control were added to incubate at room temperature (RT) in the dark for 15 minutes. Then treated the blood sample by 500μl of hemolysin at RT in the dark for 15 minutes. Then 1ml sheath solution was added to stop hemolysis. Next, blood sample was centrifuged for 5 minutes at 1000 r/min, and supernatant was discarded. Cells were washed twice by phosphate buffer saline and suspended in sheath solution. The cells were detected by flow cytometry (FACSVia, BD Biosciences).

Antibodies used for flow cytometry were obtained from BD Biosciences (San Jose, CA, USA): CD3-FITC (#340542), CD4-FITC (#340133), CD8-FITC (#340692), CD19-FITC (#340864), CD56-FITC (#340723),

FITC/PE/APC (#558517). The normal reference ranges of lymphocyte subsets were defined as follows: $CD3^+$ T cells (58.7–75%), $CD3^+CD4^+$ T cells (26.3–46.5%), $CD3^+CD8^+$ T cells (16-29.5%), NK cells (4.1–17.3%) and B cells (13.8–26.7%).

Statistical Analysis

Changes in the proportion of lymphocytes before and after 6-week induction therapy was defined as Δlymphocyte = post 6-week induction therapy lymphocyte proportion - baseline lymphocyte proportion. SPSS 22.0 statistical software and GraphPad Prism 6 statistic software were used for data analysis. Changes in the proportion of lymphocytes before and after 6-week induction therapy were compared by paired *t*-test. Associations between Δlymphocyte and clinicopathological features for LCH patients were assessed by the Pearson's χ^2 test. Survival curves were established by Kaplan-Meier method. Multivariate analysis was based on COX regression model. A two-side p value < 0.05 was considered statistically significant.

Results

Patient Characteristics

The clinicopathological features of 79 LCH patients were summarized in Table 1. Among 79 childhood LCH patients, 43 (54.4%) were male, 36 (45.6%) were female. The median age at diagnosed was 4-year-old. As for HS classification, 47 (59.5%) were single-system involvement (SS), 14 (17.7%) were multi-systemic involvement without risk organ involvement (MS RO-), 18 (22.8%) were multi-system involvement with one or more risk organs involvement (MS RO+). Considering organ involvement, 5 (6.3%) presented with bone marrow involvement, 14 (17.7%) with liver involvement, 4 (5.1%) with spleen involvement, 9 (11.4%) with lung involvement and 30 (38%) with central nervous system (CNS) involvement. 23 (29.1%) patients presented with BRAF V600E mutation, while 5 (6.3%) presented with MAP2K1 mutation. Treatment efficacy was evaluated after 6-week induction therapy, 8 (10.1%) patients were non-active disease (NAD), 40 (50.6%) were active disease-better (AD-better), 12 (15.2%) were active disease-stable (AD-stable), while 19 (24.1%) were active disease-progressive (AD-progressive).

Changes of Peripheral Blood Lymphocyte Subsets After 6-week Induction Therapy

The changes in the proportion of peripheral blood lymphocyte subsets before and after 6-week induction therapy is shown in Figure 1 and Table 2. Results showed that the percentage of $CD3^+$ T cells, $CD3^+CD4^+$ T cells and CD4/CD8 ratio were significantly increased after 6-week induction therapy (p□0.05), while the percentage of $CD3^+CD8^+$ T cells and B cells were significantly decreased after 6-week induction therapy (p□0.05). These results suggested that peripheral blood lymphocyte subsets could be influenced by induction therapy.

Correlation Between the Peripheral Blood Lymphocyte Subsets and Clinicopathological Features Before Induction Therapy

As shown in Figure 2, correlation analysis showed that $CD3^+CD8^+$ T cells and B cells before induction therapy were related to gender (r=-0.23 and 0.22). $CD3^+CD8^+$ T cells and CD4/CD8 ratio were related to age (r=0.28 and -0.32). $CD3^+$ T cells, $CD3^+CD8^+$ T cells, NK cells, B cells and CD4/CD8 ratio were related to HS classification (r=-0.34, -0.23, 0.23, 0.24 and 0.27, respectively). As for risk organs involvement, $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells and B cells were related to liver involvement (r=-0.43,-0.29,-0.25,0.23 and 0.28, respectively), $CD3^+$ T cells, $CD3^+CD8^+$ T cells, and B cells were related to bone marrow involvement (r=-0.36,-0.26 and 0.31, respectively), $CD3^+$ T cells, $CD3^+CD8^+$ T cells, B cells and CD4/CD8 ratio were related to spleen involvement (r=-0.3,-0.36,0.33 and 0.25, respectively), CD4/CD8 ratio was related to lung involvement (r=0.27). $CD3^+CD8^+$ T cells and B cells were related to BRAF V600E mutation (r=-0.23 and 0.25), $CD3^+CD8^+$ T cells and B cells were also related to MAP2K1 mutation (r=-0.24 and 0.28).

The above results suggested that $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells and B cells at baseline might be indicators for liver involvement, $CD3^+$ T cells, $CD3^+CD8^+$ T cells, and B cells may be predictors for bone marrow involvement, $CD3^+$ T cells, $CD3^+CD8^+$ T cells, B cells and CD4/CD8 ratio may act as predictors for spleen involvement, while CD4/CD8 ratio could act as indicators for lung involvement. Moreover, $CD3^+CD8^+$ T cells and B cells might be closely related to BRAF V600E and MAP2K1 mutation.

Correlation Between the Changes of Peripheral Blood Lymphocyte Subsets and Clinicopathological Features After 6-week Induction Therapy

As shown in Figure 3 and Table 3, $\Delta CD3^+$ T cells, $\Delta CD3^+CD4^+$ T cells, $\Delta CD3^+CD8^+$ T cells, ΔNK cells and ΔB cells were related to treatment efficacy($p<0.05$). As for organ involvements, $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were related to liver involvement, ΔCD4/CD8 ratio was related to CNS involvement($p<0.05$). Moreover, $\Delta CD3^+$ T cells and ΔB cells were related to BRAF V600E mutation, while $\Delta CD3^+CD4^+$ T cells was related to MAP2K1 mutation ($p<0.05$). These results suggested that treatment efficacy, liver involvement, CNS involvement, BRAF V600E and MAP2K1 mutation might affect peripheral blood lymphocyte subsets after 6-week induction therapy.

Prognostic Factors

During follow-up, 34 patients presented with disease progression, the median PFS was 19 months, while 5 patients died, the median OS was 29 months. As shown in Table 4, HS classification, liver involvement, lung involvement, BRAF V600E and MAP2K1 mutation were predictive factors for PFS ($p<0.05$). Patients

with SS disease, without liver and lung involvement, BRAF V600E and MAP2K1 wild type predicted superior PFS. Also, BRAF V600E and MAP2K1 wild type predicted superior OS (p□0.05).

Kaplan–Meier survival curves for PFS and OS with respect to Δlymphocytes were shown in Figure 4-5 and Table5. As shown in Figure 4 and Table 5, increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD4^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells, decreased ΔNK cells and increased ΔB cells were predictors for superior PFS (p□0.05). As shown in Figure 5 and Table 5, increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells and decreased ΔNK cells were predictors for superior OS (p□0.05).

Multivariate Cox regression analysis, including HS classification, liver involvement, lung involvement, BRAF V600E mutation, MAP2K1 mutation, $\Delta CD3^+$ T cells, $\Delta CD3^+CD4^+$ T cells, $\Delta CD3^+CD8^+$ T cells, ΔNK cells and ΔB cells, showed that $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were independent prognostic factors for PFS (Table 6, p□0.05). However, multivariate Cox regression analysis including the above variables showed no independent predictors for OS.

Discussion

LCH is an inflammatory myeloid neoplasm, the clinical manifestations are heterogeneous, ranging from single lesions to systemic lesions with or without risk organs involvement[11]. The outcome of LCH varies, finding prognostic factors is an urgent need. In the present study, we investigated the prognostic values of $\Delta CD3^+$ T cells, $\Delta CD3^+CD4^+$ T cells, $\Delta CD3^+CD8^+$ T cells, ΔNK cells, ΔB cells and ΔCD4/CD8 ratio in childhood LCH. Increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD4^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells, decreased ΔNK cells and increased ΔB cells were predictors for superior PFS, while increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells and decreased ΔNK cells were predictors for superior OS. Multivariate Cox regression analysis showed that $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were independent prognostic factors for PFS.

LCH is thought to be granulomatous lesions consisting of pathologic “Langerhans cells”, and immune cells such as lymphocytes, eosinophils and macrophages. Pathological features of LCH support that LCH is a reactive immune disorder, neoplastic disorder, or combination of both[1]. With the advance of genomic sequencing techniques, mitogen-activated protein kinase (MAPK) pathway is found to be activated in almost all the LCH patients[12]. In 2010, Rollins and colleagues reported recurrent somatic *BRAF V600E* mutations in 57% LCH lesions[13]. Besides *BRAF V600E*, about 20% presented with *MAP2K1* mutation, and rare presented with *ARAF* and *MAP3K1* mutations[14]. Previous studies showed *BRAF V600E* and *MAP2K1* mutations were correlated with poor prognosis in several tumors. In colorectal cancer with liver metastasis, patients with *BRAF V600E* mutation presented with shorter PFS, DFS, and higher risk of recurrence[15]. In stage □ colorectal cancer, patients with *BRAF V600E* mutation presented with worse OS, and corelated with liver metastasis[16]. In splenic diffuse red pulp small B-cell lymphoma, *MAP2K1* mutation was corelated with aggressive disease and shorter PFS[17]. In our study, 29.1% patients

presented with *BRAF V600E* mutation, while 6.3% presented with *MAP2K1* mutation, *BRAF V600E* and *MAP2K1* mutations predicted inferior PFS and OS, which was consistent with the previous studies.

Immune microenvironment could affect the development of cancers[18]. Secretion of cytokines, chemokines, changing of immune cells such as peripheral blood cells and peripheral lymphocyte subsets, or other factors, may change the status of immune microenvironment[19–22]. The peripheral lymphocyte subsets consist of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, NK cells, B cells, etc., and there are increasing evidences that peripheral lymphocyte subsets play key roles in predicting the prognosis of cancers. The examination of peripheral lymphocyte subsets is non-invasive and easy to be done, that makes them promising biomarkers for prognostic predictors for cancers.

In the present study, $\Delta CD3^+$ T cells and ΔB cells were related to *BRAF V600E* mutation, while $\Delta CD3^+CD4^+$ T cells was related to *MAP2K1* mutation (p 0.05). *BRAF V600E* and *MAP2K1* mutations were reported to be correlated with poor survival of several types of cancer, previous studies and the present study showed that *BRAF V600E* and *MAP2K1* mutations predicted poor PFS and OS in childhood LCH. The changes of peripheral lymphocyte subsets correlated with prognosis of LCH in children.

We also found that increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD4^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells, decreased ΔNK cells and increased ΔB cells were predictors for superior PFS, while increased $\Delta CD3^+$ T cells, decreased $\Delta CD3^+CD8^+$ T cells and decreased ΔNK cells were predictors for superior OS, moreover, $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells were independent prognostic factors for PFS. The prognostic values of peripheral lymphocyte subsets have been reported in many types of cancers. In advanced non-small cell lung cancer treated with immunotherapy, higher levels of $CD3^+$, $CD4^+$, and $CD8^+$ T cells but lower levels of NK cells at baseline predicted longer OS[23]. In limited stage small cell lung cancer, univariate and multivariate analyses showed that prophylactic cranial irradiation, high percentages of $CD4^+CD45RA^+$, $CD8^+CD38^+$ T cells after chemoradiotherapy independently predicted superior PFS[24]. In gastrointestinal cancer, decline in $CD4^+$ T cells after the first cycle of immunotherapy was an independent prognostic factor for OS, and could also act as an indicator for progression in patients with deficient mismatch repair/microsatellite instability-high[25]. LCH is pathologically surrounding by immune cells, but little is known about the correlation between immune cells and LCH, our previous study showed that peripheral lymphocyte subsets were closely related with the prognosis of LCH, that may provide novel predictors for LCH in children.

In summary, our study showed that peripheral lymphocyte subsets could predict the prognosis of LCH in children, and $\Delta CD3^+$ T cells and $\Delta CD3^+CD8^+$ T cells might be promising predicting biomarkers. However, there were limitations in the present study. First, this was a retrospective study enrolling small sample size in single center. Second, investigation of peripheral lymphocyte subsets could be influenced by various factors including physiological factors, that may bias the findings. Further studies are needed to confirm the value of peripheral lymphocyte subsets in childhood LCH.

Declarations

Competing Interests

None of the authors had any conflicts of interest to declare.

Author Contributions

All authors contributed to the study conception and design. YLC conceived and designed the study. FYH, WLY and YY collected the data. FYH and WLY analyzed the data and wrote the paper. All the authors read and approved the manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The studies involving human participants were reviewed and approved by the Ethics Committee of Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

References

1. Allen C E, Merad M, Mcclain K L. Langerhans-Cell Histiocytosis. N Engl J Med. 2018. 379(9), 856-868.
2. Suh J K, Kang S, Kim H, et al. Recent advances in the understanding of the molecular pathogenesis and targeted therapy options in Langerhans cell histiocytosis. Blood research. 2021. 56(S1), S65-s69.
3. Héritier S, Emile J F, Hélias-Rodzewicz Z, et al. Progress towards molecular-based management of childhood Langerhans cell histiocytosis. Arch Pediatr. 2019. 26(5), 301-307.
4. Héritier S, Emile J F, Barkaoui M A, et al. BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy. J Clin Oncol. 2016. 34(25), 3023-3030.
5. Hayase T, Saito S, Shioda Y, et al. Analysis of the BRAF and MAP2K1 mutations in patients with Langerhans cell histiocytosis in Japan. Int J Hematol. 2020. 112(4), 560-567.
6. Thorsson V, Gibbs D L, Brown S D, et al. The Immune Landscape of Cancer. Immunity. 2018. 48(4), 812-830.e814.
7. Dai S, Ren P, Ren J, et al. The Relationship between Lymphocyte Subsets and the Prognosis and Genomic Features of Lung Cancer: A Retrospective Study. Int J Med Sci. 2021. 18(10), 2228-2234.
8. Yang J, Xu J, E Y, et al. Predictive and prognostic value of circulating blood lymphocyte subsets in metastatic breast cancer. Cancer medicine. 2019. 8(2), 492-500.

9. Marchi F, Missale F, Incandela F, et al. Prognostic Significance of Peripheral T-Cell Subsets in Laryngeal Squamous Cell Carcinoma. Laryngoscope investigative otolaryngology. 2019. 4(5), 513-519.
10. Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. Blood. 2013. 121(25), 5006-5014.
11. Abla O, Rollins B, Ladisch S. Langerhans cell histiocytosis: progress and controversies. Br J Haematol. 2019. 187(5), 559-562.
12. Bh. D, E. L R, J. P, et al. Activating mutations in CSF1R and additional receptor tyrosine kinases in histiocytic neoplasms. Nat Med. 2019. 25(12), 1839-1842.
13. Badalian-Very G, Vergilio J A, Degar B A, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood. 2010. 116(11), 1919-1923.
14. Brown N A, Furtado L V, Betz B L, et al. High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans cell histiocytosis. Blood. 2014. 124(10), 1655-1658.
15. Margonis G A, Buettner S, Andreatos N, et al. Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer. JAMA surgery. 2018. 153(7), e180996.
16. Shimada Y, Muneoka Y, Nagahashi M, et al. BRAF V600E and SRC mutations as molecular markers for predicting prognosis and conversion surgery in Stage IV colorectal cancer. Sci Rep. 2019. 9(1), 2466.
17. Martinez D, Navarro A, Martinez-Trillos A, et al. NOTCH1, TP53, and MAP2K1 Mutations in Splenic Diffuse Red Pulp Small B-cell Lymphoma Are Associated With Progressive Disease. Am J Surg Pathol. 2016. 40(2), 192-201.
18. Lei X, Lei Y, Li J K, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer Lett. 2020. 470, 126-133.
19. Hinshaw D C, Shevde L A. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res. 2019. 79(18), 4557-4566.
20. Ozga A J, Chow M T, Luster A D. Chemokines and the immune response to cancer. Immunity. 2021. 54(5), 859-874.
21. Ferrari S M, Fallahi P, Galdiero M R, et al. Immune and Inflammatory Cells in Thyroid Cancer Microenvironment. International journal of molecular sciences. 2019. 20(18),
22. Farhood B, Najafi M, Mortezaee K. CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: A review. J Cell Physiol. 2019. 234(6), 8509-8521.
23. Ottonello S, Genova C, Cossu I, et al. Association Between Response to Nivolumab Treatment and Peripheral Blood Lymphocyte Subsets in Patients With Non-small Cell Lung Cancer. Front Immunol. 2020. 11, 125.
24. Chen Y, Jin Y, Hu X, et al. Effect of chemoradiotherapy on the proportion of circulating lymphocyte subsets in patients with limited-stage small cell lung cancer. Cancer Immunol Immunother. 2021.

70(10), 2867-2876.
25. Liu C, Wang Y, Li S, et al. Early change in peripheral CD4(+) T cells associated with clinical outcomes of immunotherapy in gastrointestinal cancer. Immunotherapy. 2021. 13(1), 55-66.

Tables

Table 1

Clinicopathological features of patients (n=79)

Characteristics	Number (%)
Gender	
Male	43 (54.4%)
Female	36 (45.6%)
Age	
Median age at diagnosis	4-year-old
HS classification	
SS	47 (59.5%)
MS RO-	14 (17.7%)
MS RO+	18 (22.8%)
Involvement	
Bone marrow	5 (6.3%)
Liver	14 (17.7%)
Spleen	4 (5.1%)
Lung	9 (11.4%)
CNS	30 (38%)
BRAF V600E	
Mutation	23 (29.1%)
Wild type	56 (70.9%)
MAP2K1	
Mutation	5 (6.3%)
Wild type	74 (93.7%)
Treatment efficacy after 6-week induction therapy	
NAD	8 (10.1%)
AD-better	40 (50.6%)
AD-stable	12 (15.2%)
AD-progressive	19 (24.1%)

Table 2

Changes of peripheral blood lymphocyte subsets after 6-week induction therapy

Lymphocyte subsets	Percentage (median)		*p* value
	Before	After	
$CD3^+$ T cells	67.51	70.12	**0.043**
$CD3^+CD4^+$ T cells	34.03	35.75	**0.023**
$CD3^+CD8^+$ T cells	27.16	23.17	**0.027**
NK cells	9.23	9.9	0.621
B cells	18.2	15.43	**0.002**
CD4/CD8 ratio	1.37	1.59	**0.019**

Table 3

Correlation between the changes of peripheral blood lymphocyte subsets and clinicopathological features after 6-week induction therapy

Variables	Treatment efficacy	Liver involved	CNS involved	*BRAF V600E*	*MAP2K1*
	p value	*p* value	*p* value	*p* value	*p* value
$\Delta CD3^+$ T cells	**0.001**	**0.046**	0.693	**0.018**	0.064
$\Delta CD3^+CD4^+$ T cells	**0.004**	0.059	0.432	0.926	**0.04**
$\Delta CD3^+CD8^+$ T cells	**0.008**	**0.032**	0.124	0.082	0.509
ΔNK cells	**0.001**	0.959	0.079	0.249	0.179
ΔB cells	**0.004**	0.929	0.478	**0.004**	0.051
ΔCD4/CD8 ratio	0.282	0.082	**0.016**	0.778	0.27

Table 4

Association between clinicopathological features and prognosis

Characteristics	Median PFS (months)	*p* value	Median OS (months)	*p* value
Gender		0.653		0.259
Male	31		Not reached	
Female	Not reached		Not reached	
Age		0.062		0.874
Median age at diagnosis	18		Not reached	
HS classification		**0.001**		0.651
SS	Not reached		Not reached	
MS RO-	Not reached		Not reached	
MS RO+	10		Not reached	
Involvement				
Bone marrow	12	0.071	Not reached	0.216
Liver	10	**0.001**	Not reached	0.221
Spleen	3	0.121	Not reached	0.157
Lung	12	**0.003**	Not reached	0.644
CNS	27	0.068	Not reached	0.249
BRAF V600E		**0.008**		**0.006**
Mutation	19		Not reached	
Wild type	Not reached		Not reached	
MAP2K1		**0.033**		**0.001**
Mutation	14		Not reached	
Wild type	Not reached		Not reached	

Table 5

Univariate analysis of the changes of peripheral blood lymphocyte subsets associated with PFS and OS

Variables	Median PFS (months)	*p* value	Median OS (months)	*p* value
ΔCD3$^+$ T cells		**0.001**		**0.013**
Increased	Not reached		Not reached	
Decreased	11.5		Not reached	
ΔCD3$^+$CD4$^+$ T cells		**0.013**		0.256
Increased	26		Not reached	
Decreased	Not reached		Not reached	
ΔCD3$^+$CD8$^+$ T cells		**0.001**		**0.029**
Increased	14		Not reached	
Decreased	Not reached		Not reached	
ΔNK cells		**0.002**		**0.041**
Increased	24		Not reached	
Decreased	Not reached		Not reached	
ΔB cells		**0.020**		0.068
Increased	Not reached		Not reached	
Decreased	27		Not reached	
ΔCD4/CD8 ratio		0.475		0.082
Increased	36		Not reached	
Decreased	31		Not reached	

Table 6

Multivariate analysis of prognostic factors affecting PFS

Variables	PFS		
	HR	95% CI	*p* value
HS classification	1.85	0.819-4.18	0.139
Liver involvement	2.266	0.43-11.941	0.335
Lung involvement	0.762	0.19-3.06	0.701
BRAF V600E	0.543	0.204-1.442	0.22
MAP2K1	0.442	0.089-2.208	0.32
ΔCD3$^+$ T cells	7.971	2.835-22.409	**0.001**
ΔCD3$^+$CD4$^+$ T cells	0.621	0.232-1.662	0.343
ΔCD3$^+$CD8$^+$ T cells	0.365	0.136-0.98	**0.046**
ΔNK cells	0.433	0.155-1.21	0.111
ΔB cells	1.493	0.433-5.15	0.526

Figures

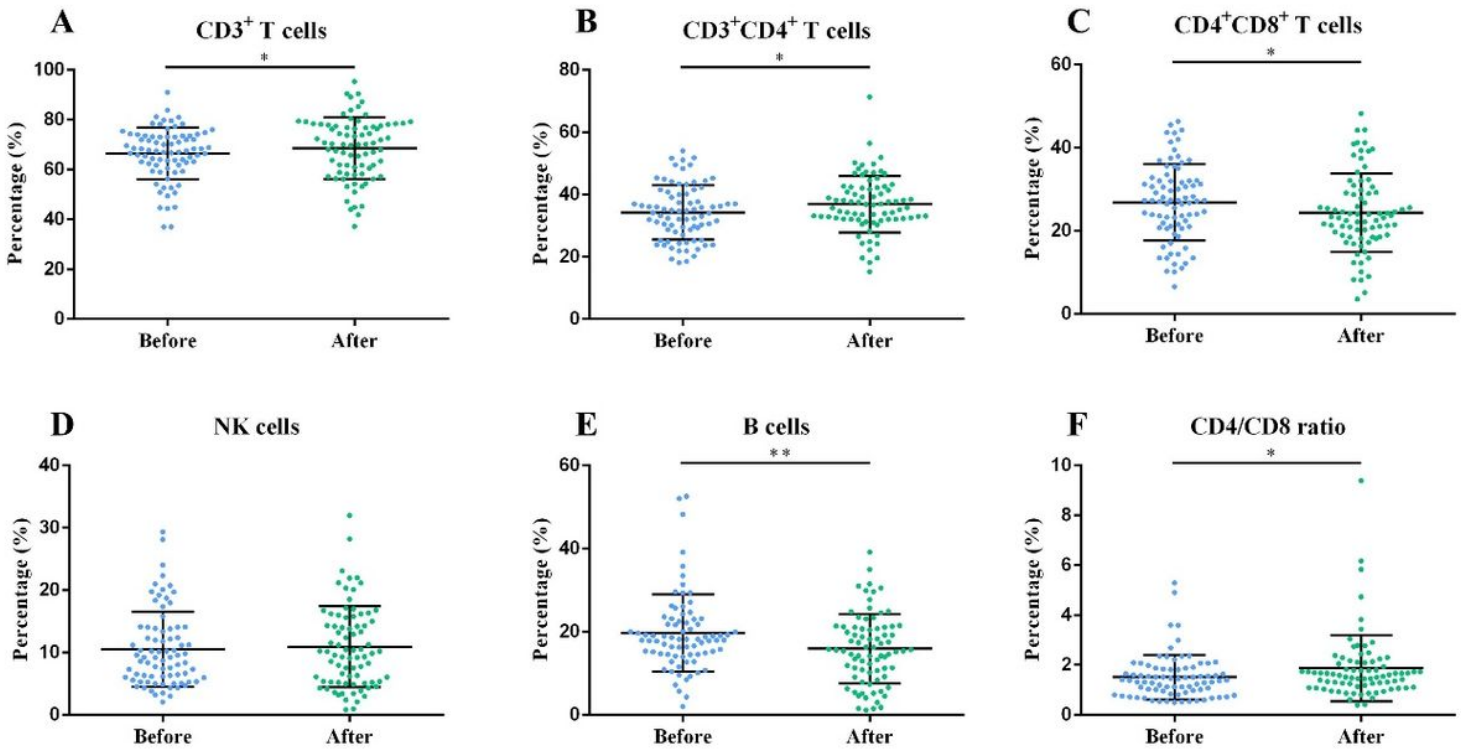

Figure 1

Changes of peripheral blood lymphocyte subsets after 6-week induction therapy. Percentages of peripheral blood lymphocyte subsets including (A) CD3+ T cells, (B) CD3+CD4+ T cells, (C) CD3+CD8+ T cells, (D) NK cells, (E) B cells and (F) CD4/CD8 ratio before and after 6-week induction therapy were analyzed.

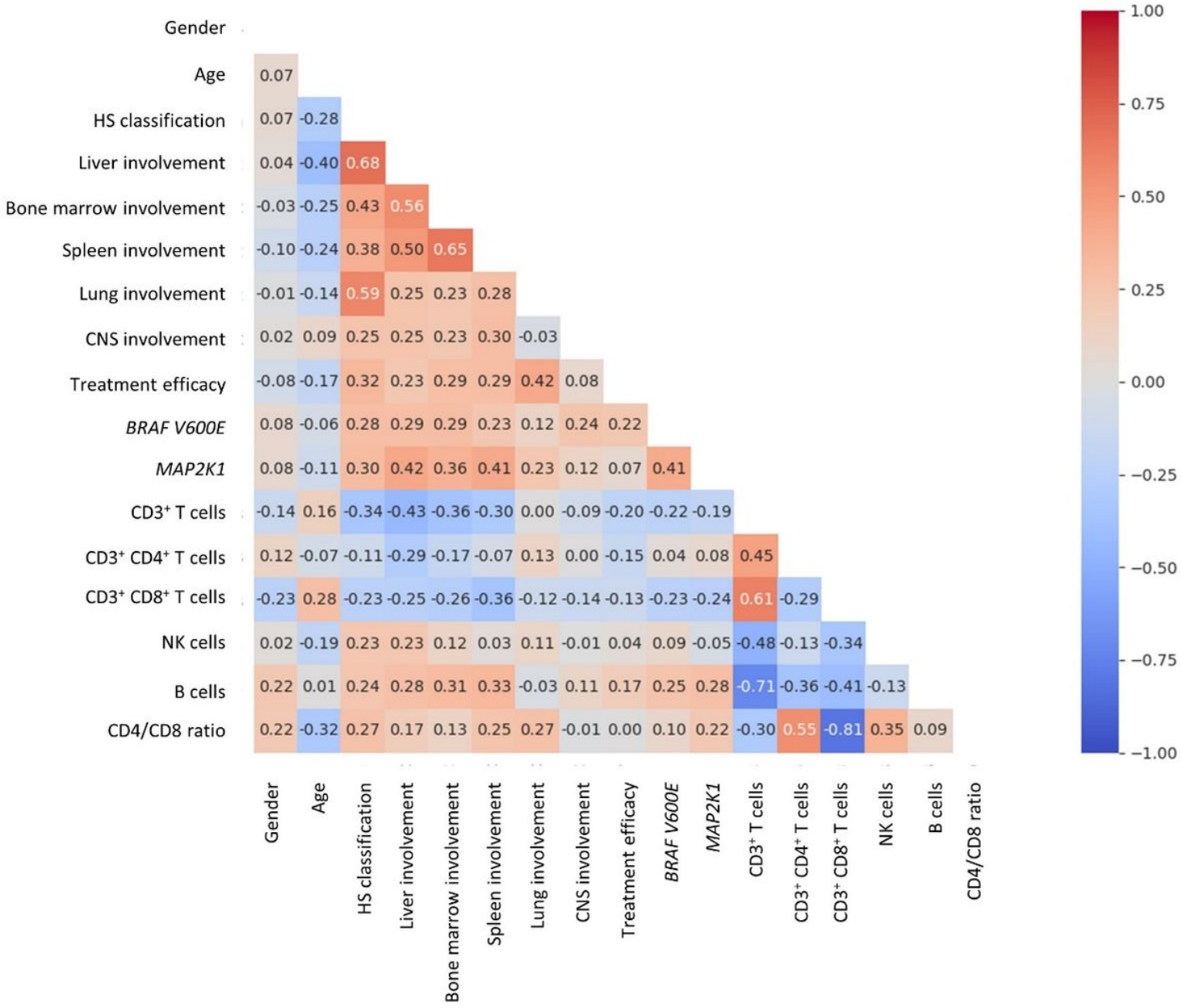

Figure 2

The correlation heat map between peripheral blood lymphocyte subsets and clinicopathological features before 6-week induction therapy.

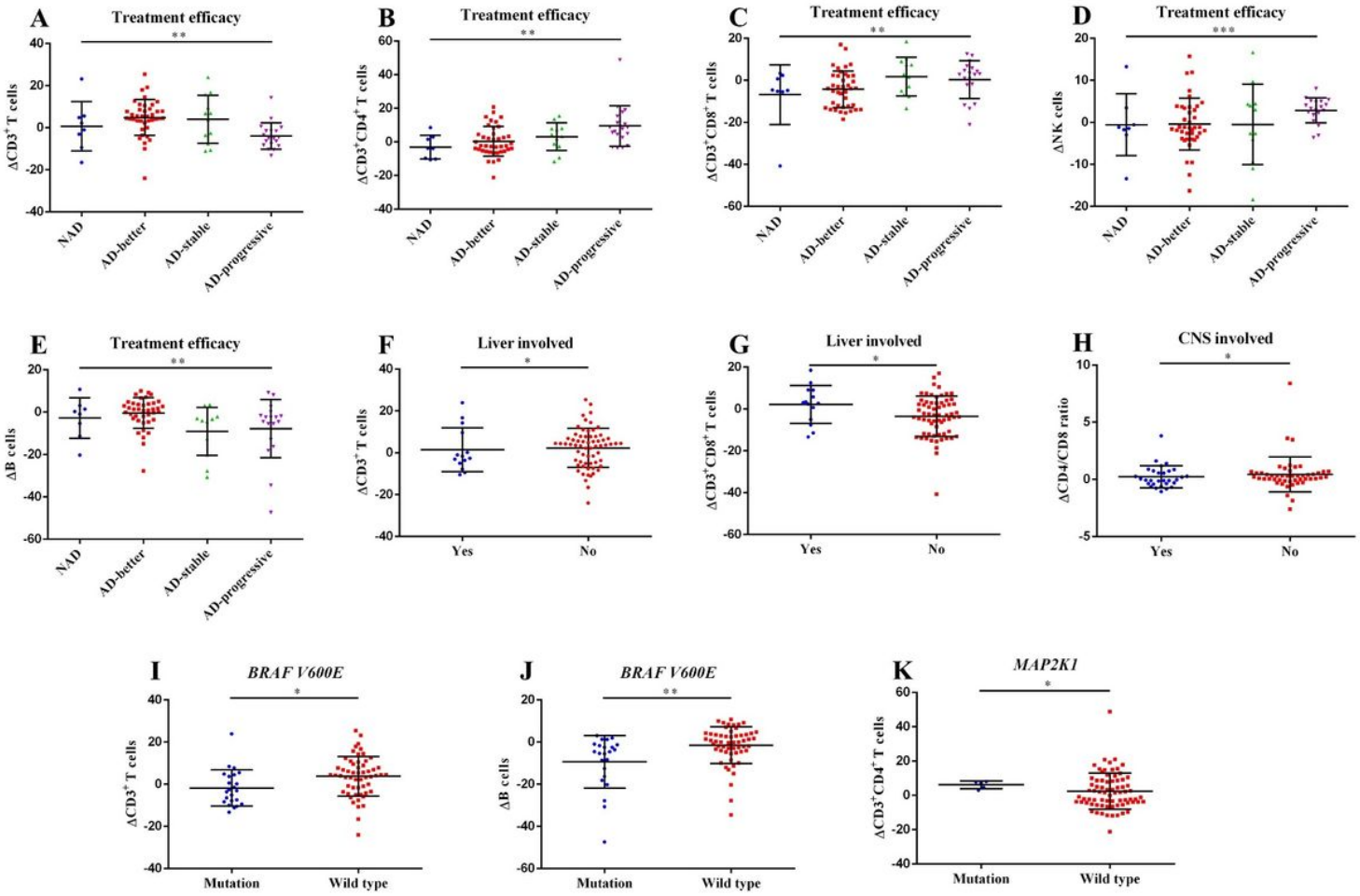

Figure 3

Correlation between the changes of peripheral blood lymphocyte subsets and clinicopathological features after 6-week induction therapy. (A-E) The correlation between the A) ΔCD3+ T cells, B) ΔCD3+CD4+ T cells, C) Δ CD3+CD8+ T cells, D) ΔNK cells and E) ΔB cells and treatment efficacy. (F-G) The correlation between F) ΔCD3+ T cells, G) ΔCD3+CD8+ T cells and liver involvement. (H) The correlation between ΔCD4/CD8 ratio and CNS involvement. (I-J) The correlation between I) Δ CD3+ T cells, J) ΔB cells and *BRAF V600E* mutation. (K) The correlation between ΔCD3+CD4+ T cells and *MAP2K1* mutation.

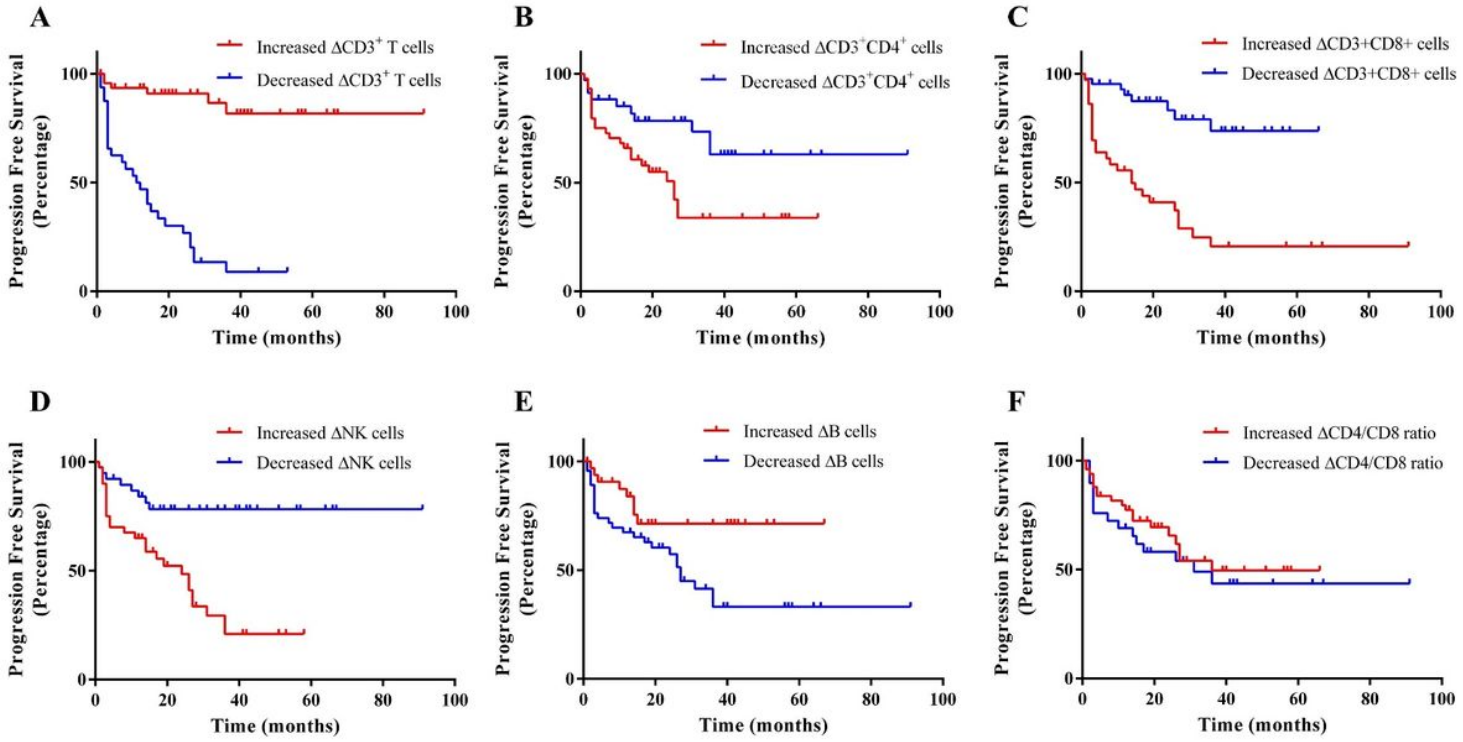

Figure 4

Kaplan–Meier survival curves for PFS. Kaplan–Meier curves for PFS according to (A) ΔCD3+ T cells, (B) ΔCD3+CD4+ T cells, (C) Δ CD3+CD8+ T cells, (D) ΔNK cells, (E) ΔB cells and (F) ΔCD4/CD8 ratio.

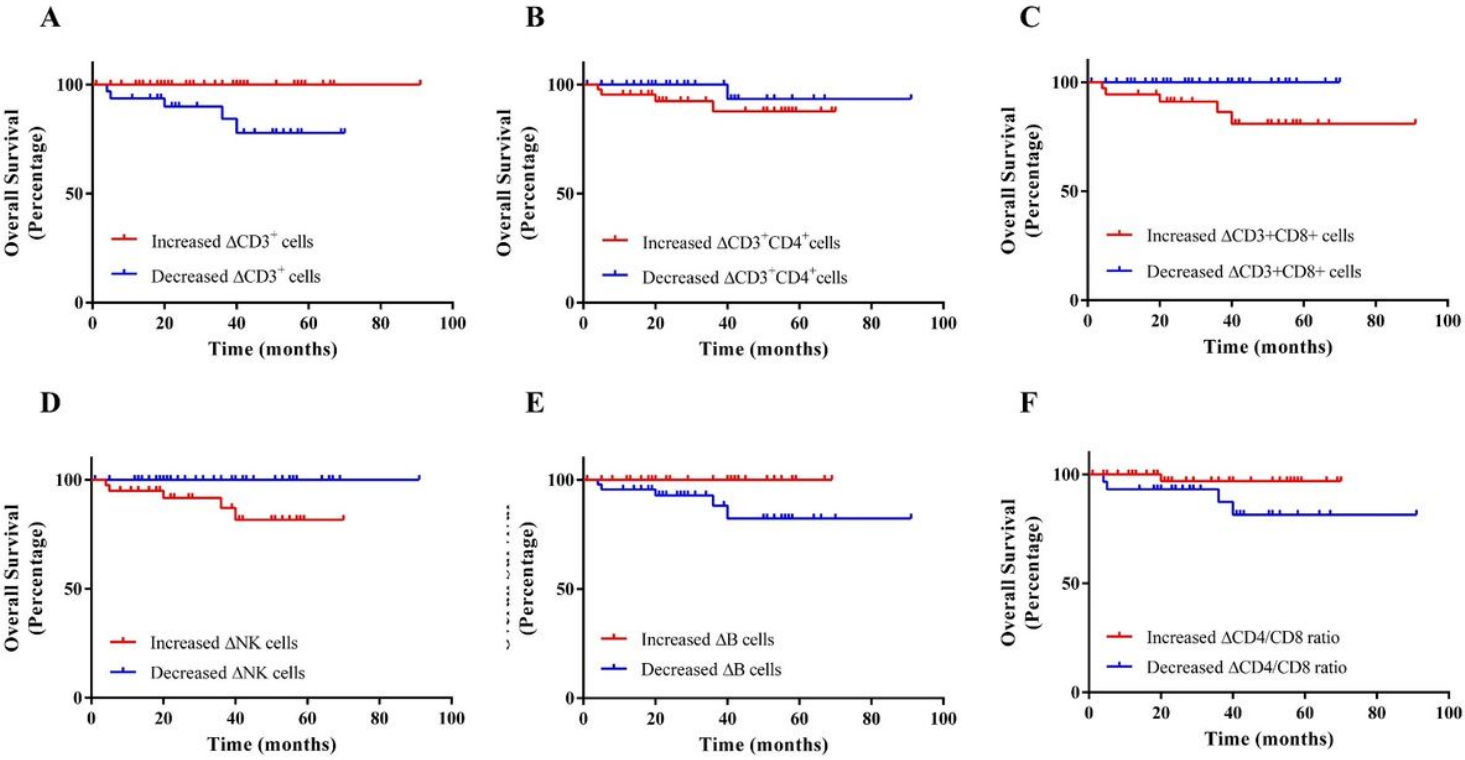

Figure 5

Kaplan–Meier survival curves for OS. Kaplan–Meier curves for OS according to (A) ΔCD3+ T cells, (B) ΔCD3+CD4+ T cells, (C) Δ CD3+CD8+ T cells, (D) ΔNK cells, (E) ΔB cells and (F) ΔCD4/CD8 ratio.