Prognostic Significance of Absolute Lymphocyte Count and Lymphocyte Subsets in Lymphomas

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Abstract: Lymphocytopenia is a poor prognostic marker in initial staging of non-Hodgkin lymphomas (below 1.0×10^9/l) and Hodgkin lymphoma (below 0.6×10^9/l) and in relapsed diffuse large B cell lymphoma. Early lymphocyte recovery $\geq0.5\times10^9$/l after autologous and allogeneic stem cell transplantation is a significant predictor of tumor control and survival in lymphomas. Natural killer cells are involved in tumor cell killing and are the only subset of lymphocytes associated with disease outcome in initial staging and after autologous stem cell transplantation in lymphomas. The antitumor effect of various NK cell subsets should be defined.

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Introduction

Broader understanding of lymphoma biology and host immune system led the investigators to look for new prognostic markers that could better predict disease outcome in the era of immuno-chemotherapy. Novel molecular markers have been introduced as independent predictors of outcome in lymphomas, but their applicability in routine practice is still limited due to technical and financial demands.

Lymphocytes are not only targets for immuno-chemotherapy, but they play active role in tumor control. The examination of absolute lymphocyte counts and lymphocyte subsets in peripheral blood is standardized and financially manageable. This review summarizes the prognostic impact of absolute lymphocyte counts and lymphocyte subsets on lymphoma outcome in initial staging, relapse and after autologous or allogeneic stem cell transplantation.

Lymphocyte count and lymphocyte subsets in initial staging of lymphomas

Absolute lymphocyte count (ALC) below 1.0×10^9/l is a poor prognostic marker in newly diagnosed diffuse large B-cell lymphoma (DLBCL) and is associated with shorter overall survival (OS) and progression-free survival (PFS) (Oki et al., 2008). The adverse prognostic impact of lymphocytopenia $<1.0\times10^9$/l on overall survival was also observed at diagnosis of follicular lymphomas grade 1 and 2 (Siddiqui et al., 2006). The mechanism of lymphocytopenia is unknown. In the study of Oki it was associated with advanced stage, poor performance status, B symptoms, elevated beta2microglobulin and higher IPI risk group. Addition of rituximab improved OS in patients with low lymphocyte counts but not in those with high counts (Oki et al., 2008). In the rituximab era the international prognostic index (IPI) can no longer identify a group of patients with a less than 50% chance of survival (Sehn et al., 2007). Cox et al. proposed a new prognostic score in DLBCL, that combines absolute lymphocyte counts and revised international prognostic score (ALC/R-IPI): (1) low risk: R-IPI = very good or good and ALC $<0.84\times10^9$/l; (2) intermediate risk: patients with at least one risk factor (R-IPI = poor or ALC $<0.84\times10^9$/l); (3) high risk: patients with both risk factors (Cox et al., 2008). This new prognostic score was highly significant for OS, PFS and event-free survival (EFS).

Plonquet et al. prospectively analyzed prognostic value of ALC and lymphocyte subsets in DLBCL with age adjusted IPI 2-3. ALC did not correlate with disease outcome in this study. Natural killer (NK) cell counts were the only lymphocyte subset being significantly associated with disease outcome: absolute count of NK cells $<80/\mu$l was predictive of no response and of shorter EFS (Plonquet et al., 2007). NK cells deficiency could be a potential marker of disease severity, but lymphocyte subset counts were not associated with bone marrow involvement or tumor burden. NK cells are important effectors of antitumor immunity, they play a critical role in innate immune surveillance of B cell lymphomas (Street et al., 2004). Circulating NK cells are divided into two subsets: $CD56^{high}CD16^{neg}$ NK cells predominates in lymph nodes and have little cytolytic activity, $CD56^{low}$

$CD16^+$ NK cells predominate in peripheral blood and inflamed tissues and display potent cytotoxicity. The antitumor effect of various NK cell subsets should be defined: NK cells expressing activating receptors (NCR, NKG2D), coactivating receptors (2B4, DNAM-1) and inhibiting receptors (CD94 and KIR molecules) (Moretta et al., 2008). NK cells are the principal cells in peripheral blood that kill rituximab-opsonized B cells. Experimental studies confirmed, that rituximab-based immunotherapy of cancer may be enhanced by means of infusion of compatible NK cells and inhibition of monocyte shaving activity (Beum et al., 2008).

Patients with newly diagnosed chronic lymphocytic leukaemia and a higher peripheral blood T-cells and NK-cells to malignant monoclonal B-cell ratio showed delayed time to treatment (Palmer et al., 2008).

ALC below 0.6×10^9/l in initial staging of advanced Hodgkin lymphoma (HL) is an independent prognostic factor incorporated in the international prognostic score (Hasenclever and Diehl, 1998). Low number of lymphocytes is a marker of immunological dysregulation due to cytokine release by Hodgkin lymphoma cells. Prognostic impact of lymphocyte subsets in HL at diagnosis awaits further investigation.

Lymphocyte count and lymphocyte subsets in relapsed lymphomas

ALC predicts survival in the first relapse of DLBCL, suggesting that the host immunity is an important variable associated with the outcome of treatment (Porrata et al., 2009). In this study DLBCL patients in the first relapse with ALC $\geq1.0\times10^9$/l experienced superior OS and PFS. In addition, higher ALC-R demonstrated superior survival regardless whether patients underwent autologous stem cell transplantation (ASCT) or not. Multivariate analysis identified ALC as an independent predictor of survival in first relapse of DLBCL patients. The limitation of the study was, that authors did not analyze lymphocyte subpopulations and the presence of malignant cells could spuriously increase the number of lymphocytes. Additionally, patients were not randomly assigned to ASCT versus other therapies and there could be a potential bias in selection of patient undergoing ASCT. The prognostic impact of ALC in other types of relapsed non-Hodgkin lymphomas (NHL) and HL is under investigation.

Lymphocyte recovery post-autologous stem cell transplantation in lymphomas

High dose chemotherapy with ASCT is an effective treatment for patients with relapsed lymphomas. Early lymphocyte recovery after ASCT at day +15 seems to be a significant predictor for tumor control and survival in hematologic malignancies including NHL and HL (Porrata et al., 2002a, b, 2008; Gordan et al., 2003; Boulassel et al., 2006; Kim et al., 2006). Gordan et al. demonstrated that ALC $\geq0.667\times10^9$/l at day +15 after ASCT independently predicts better PFS in NHL and HL (Gordan et al., 2003). Others reported significantly better clinical outcome in NHL patients

with ALC $\geq 0.5 \times 10^9$/l at day +15 post-transplant (Porrata et al., 2001a; Yoong et al., 2005; Joao et al., 2006). An explanation for the survival advantage associated with ALC recovery after ASCT is the possibility that early immune reconstitution may have a protective effect against residual disease progression.

Sources involved in the ALC recovery are: CD34 stem cells, reinfused graft lymphocytes, host stem cells and host lymphocytes surviving high dose conditioning chemotherapy.

Seshadri et al. reported 146 patients with relapsed/refractory HL who underwent ASCT and found no association between ALC at day +15 and at day +90 with PFS, only lymphocyte counts at apheresis were predictive of PFS (Seshadri et al., 2008). Preapheresis ALC is directly dependent on the time interval from last myelosuppressive chemotherapy (Holtan et al., 2006). The number of re-infused NK cells in the apheresis product significantly affected ALC recovery early post-transplant, but T and B cells did not correlate with ALC post-ASCT (Porrata et al., 2003).

Reported data suggest that $CD34^+$ cell count is predictive for kinetics of lymphocyte recovery after ASCT and is an independent prognostic factor for OS and EFS after ASCT in patients with NHL (Yoon et al., 2009). Engraftment does not necessarily correlate with total $CD34^+$ cell numbers, only with subsets of $CD34^+$ cells: $CD34^+Lin^-Selectin^+$ is the best predictor of engraftment rapidity and $CD34^+Thy\text{-}1^+$ correlates with durable engraftment (Pratt et al., 2001).

Recovery of lymphocyte subsets after ASCT: $CD3^+$ cells returns to normal after 2 years in HL and NHL. $CD4^+$ subset achieves reference values during the sixth year, $CD8^+$ and $CD19^+$ lymphocytes recover at day 60 and at day 120 respectively. $CD16^+CD56^+$ and $CD3^+$/HLA DR^+ lymphocytes recovered starting from day 30 (Laurenti et al., 2004).

NK cell numbers day +15 after ASCT have been reported as an independent predictor for survival in NHL (Porrata et al., 2008).

Lymphocyte recovery post-allogeneic stem cell transplantation in lymphomas

Faster lymphocyte recovery (ALC $\geq 0.5 \times 10^9$/l) correlated with better survival in allogeneic blood cell transplantation compared with allogeneic bone marrow transplantation (Pavletic et al., 1998).

T-helper cell reconstitution after non-myeloablative allogeneic stem cell transplantation is significantly faster than after conventional myeloablative conditioning. Recovery of B cells is faster after conventional transplantation (Schulenburg et al., 2005). Faster recovery of IgM levels was observed after non-myeloablative transplantation and a delayed recovery of IgA levels was observed in both groups. In patients receiving rituximab 1–12 months before allogeneic stem cell transplantation B-cell counts and immunoglobulin levels were reduced for up to 24 months following transplantation (Buser et al., 2008).

NK cells have potent antitumor activity according to preclinical studies and the effect of IL-2 in combination with IFN-alpha has been studied to up-regulate NK-cell cytotoxicity (Porrata et al., 2001b). T cells coexpressing natural killer cell proteins (NKT) include a CD1d-reactive subset that influences immunity by rapidly producing large amounts of Th1 and/or Th2 cytokines. Th1 CD1d-reactive NKT cells could stimulate antitumor responses and suppress graft-vs-host disease in allogeneic stem cell transplantation (Shaulov et al., 2008).

Conclusion

New biomarkers and absolute lymphocyte counts including lymphocyte subsets should be taken into consideration when new prognostic scoring systems in lymphomas are created. These new prognostic scores should be defined and tested on large cohorts of patients at diagnosis, in relapse and following stem cell transplantation.

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