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Review

Immunomonitoring and prognostic relevance of neutrophils in clinical trials

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ABSTRACT

The clinical relevance of the interaction between human cancer and neutrophils has recently begun to emerge. This review will focus on recently published articles regarding immunomonitoring of neutrophils in blood and tumor tissue in clinical trials comprising the main human tumor types, with a strong emphasis on independent prognostic relevance assessed by multivariate analyses.

The prognostic role of tumor-infiltrating neutrophils, elevated blood neutrophils and elevated blood neutrophil/lymphocyte ratio has been associated with poor clinical outcome in several human cancers, most notably in renal cell carcinoma, melanoma, colorectal cancer, hepatocellular carcinoma, cholangiocarcinoma, glioblastoma, GIST, gastric, esophageal, lung, ovarian and head and neck cancer. A striking finding is the notion that high baseline neutrophil count in either tumor or blood, or both, was identified as strong, independent risk factor for poor outcome in multivariate analyses, and the negative prognostic impact of neutrophils was not eliminated by increasing the dose of cytokines, chemotherapy, or targeted therapy. For several cancers, patients benefit most from therapy if baseline neutrophil was low. Thus, baseline neutrophils over-ride nadir counts in prognostic significance.

In summary, a proportion of patients who do not experience benefit from surgery or medical intervention may be associated with a worst prognosis because they are characterized by baseline tumor-related neutrophilia protecting them from benefit from therapy. Further research to unraveling the cancer biology and new treatment options is encouraged.

1. Introduction

Neutrophils are the most common leukocyte subset in the bloodstream. Neutrophils are short-lived white blood cells derived from bone marrow myeloid precursors. Attention to their potential role in human cancer has largely been ignored. New findings suggest that the role of neutrophils in cancer-related inflammation may need careful reappraisal of infiltration, polarization, and prognostic significance of neutrophils in human cancer [1].

Previous older studies have advocated a role of direct or antibody-dependent killing of tumor cells by neutrophils (recently reviewed in [2]). One of the oldest reports of a potential anti-tumor effect of the innate immune system comes from William Coley (reviewed in [3]). He observed that patients with febrile streptococcal infection within an ulcerated tumor had a better prognosis than patients with uninfected tumors. For obvious reasons, he was unable to explain the observations in immunology terms. Since Coley's initial reports, knowledge about mechanisms in cellular immunity has increased. However, cancer therapy for invasive human cancer utilizing a neutrophil-mediated approach has not yet been established [4].

This review will focus on recently published articles regarding immunomonitoring of neutrophils in blood and tumor tissue in clinical trials comprising the main human tumor types, with a strong emphasis on independent prognostic relevance assessed by multivariate analyses.

2. Kidney cancer

The first report of neutrophils as an adverse prognostic factor for patients with metastatic renal cell carcinoma (mRCC) was published in 1996 by Lopez Hanninen et al. [5]. In a series of 215 consecutive patients with mRCC treated with interleukin-2 (IL-2) based immunotherapy, elevated baseline blood neutrophils ($>6 \times 10^9$/L) was identified as an independent risk factor of short overall survival (OS). Subsequently, the Groupe Français d'Immunothérapie published in 2002 results comprising 782 patients in successive multicenter trials using cytokine regimens [6]. Analyses were performed on this large prospective database to identify prognostic factors for survival and predictive factors for progression. Elevated baseline neutrophil count ($>7.5 \times 10^9$/L) was independently predictive of short survival in a multivariate analysis. The authors also identified four independent factors predictive

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of rapid progression under cytokine treatment: presence of hepatic metastases, <1 year from renal tumor to metastases, more than one metastatic site, and elevated baseline neutrophil counts (>7.5 × 10^9/L). Patients who combined at least three of these factors had >80% probability of rapid progression despite treatment. The authors stated that these results should be taken into account when making the decision to treat with cytokines. The following years the research group from Aarhus University Hospital in Denmark published their translational results based on 443 serial blood samples and 225 serial tumor core biopsies obtained at baseline and during treatment with IL-2 based therapy in 120 consecutive patients with mRCC [7]. The vast majority of these patients were included in phase II trials. Based on these analyses, the authors were able to map the orchestration of immune cells in blood and tumor at baseline and during IL-2 based immunotherapy [8]. An understanding of IL-2 based immunotherapy as a "targeted therapy" requiring lymphocyte subsets for tumor rejection emerged from these analyses. In addition, an important understanding of the powerful negative impact of neutrophils also appeared. The analyses revealed that no blood lymphocyte subset was correlated with survival whereas high numbers of baseline blood neutrophils, on-treatment blood neutrophils and on-treatment blood monocytes were correlated with short survival [8]. Low numbers of on-treatment blood neutrophils were correlated with response [8]. Evaluating intra-tumoral immune cells, high numbers of baseline $CD57^+$ natural killer (NK) cells, baseline $CD4^+$ T-cells, and on-treatment $CD3^+$ T-cells were significantly correlated with favorable survival [9] whereas baseline presence of intratumoral neutrophils was correlated with short survival [10]. Thus, neutrophils and monocytes/macrophages were "bad guys" and T cells and NK-cells were "good guys" for the outcome of IL-2 based immunotherapy [7]. However, it appeared that the "bad guys" had stronger prognostic impact than the "good guys". Strikingly, in a randomized phase II trial of IL-2 alone versus IL-2 plus histamine [11], patients with high numbers of neutrophils in peripheral blood at baseline (>6 × 10^9/L) and after 8 weeks of treatments (>4.57 × 10^9/L) had very poor survival, with apparently no impact of either IL-2-alone or IL-2 plus histamine treatment, as almost all patients with high blood neutrophils were dead within 2 years from commencement of therapy [12]. Only patients with low numbers of blood neutrophils at baseline and during treatment achieved long-term survival. In another cohort of patients treated with low-dose IL-2 based immunotherapy, even lower level of blood neutrophils (≥2.19 × 10^9/L) at week 5 after commencement of therapy was associated with poor survival. Thus, patients with blood neutrophils ≥2.19 × 10^9/L had a median survival of 10.9 months whereas patients with blood neutrophils < 2.19 had a median survival of 25.1 months [8]. Based on a final multivariate analyses including baseline factors only, the authors pointed on five clinical features (performance status, bone metastases, lymph node metastases, low hemoglobin and high lactate dehydrogenase) and three supplemental immunological features (presence of intratumoral $CD66^+$ neutrophils > 0, high blood neutrophils > 6.0 × 10^9/L and low intratumoral $CD57^+$ NK cells < 50 cells/mm^2) as independent prognostic factors of survival in patients with mRCC receiving IL-2 [10]. These three independent immunological parameters had significant discriminatory power as supplemental risk factors in prognostic models based on the clinical risk factors, identifying subgroups within the favorable clinical group with estimated 5-year survival rates of 60%, 25% and 0%, respectively. The authors concluded that patients with several poor prognostic features, based on clinical risk factors only or clinical risk factors supplemented with immunologic risk factors, should not receive IL-2-based immunotherapy [10]. It is noteworthy, that these analyses – comprising the vast majority of immune cell subsets – identified baseline neutrophils, both in the blood compartment as well as in the tumor compartment, as an independent factor for short survival in patients with metastatic renal cell carcinoma, and also pointed at on-treatment blood neutrophils as a risk factor, emphasizing the compelling prognostic relevance of neutrophils. For comparison, only one lymphocyte subset (intratumoral $CD57^+$ NK cells) was identified as an independent factor for favorable survival.

In the era of targeted therapy, Choueiri et al. evaluated 120 patients with metastatic clear-cell RCC receiving bevacizumab, sorafenib, sunitinib, or axitinib on prospective clinical trials at the Cleveland Clinic [13]. Multivariate analysis identified elevated baseline neutrophil count (>4.5 × 10^9/L) as an independent, adverse prognostic factor for short progression-free survival (PFS). Heng et al. evaluated prognostic factors for OS in 645 patients with mRCC treated with vascular endothelial growth factor (VEGF)-targeted therapy [14]. Data were collected from three US and four Canadian cancer centers. In addition to well-known clinical risk features, neutrophils greater than the upper limit of normal (ULN) and platelets greater than the ULN were independent adverse prognostic factors. Patients were segregated into three risk categories: the favorable-risk group (no prognostic factors), in which 2-year OS was 75%; the intermediate-risk group (one or two prognostic factors), in which 2y OS was 53%; and the poor-risk group (three to six prognostic factors), in which 2y OS was 7%. The major contribution of this prognostic model is the addition of biological information, i.e., platelet and neutrophil counts, to the Memorial Sloan Kettering Cancer Center prognostic model, which is based on clinical features only. This model has recently been validated in an independent cohort of patients [15].

Keizman et al. evaluated the association of pre-treatment neutrophil to lymphocyte ratio (NLR) with response rate, PFS and OS in 109 patients treated with sunitinib for mRCC [16]. A low baseline blood NLR ≤ 3 was independently correlated with response to sunitinib, and independently correlated with favorable PFS and OS.

In non-metastatic, localized, clear cell RCC, the prognostic importance of intratumoral neutrophils have been assessed by Jensen et al. [17]. The study comprised 121 consecutive patients who had a nephrectomy for localized clear cell RCC. In multivariate analysis, the presence of intratumoral $CD66^+$ neutrophils was independent prognostic factors significantly associated with short recurrence-free survival, cancer-specific survival and OS. Applying the prognostic value of intratumoral neutrophils to the Leibovich low-/intermediate-risk group showed a 5-year recurrence-free survival of 53% in patients with presence of intratumoral neutrophils compared with 87% in patients with absence of intratumoral neutrophils. The estimated concordance index was 0.74 using the Leibovich risk score and 0.80 when intratumoral neutrophils were added. Thus, patients with intratumoral neutrophils should have a closer follow-up. Intratumoral neutrophils may also serve as a new stratification factor for randomized trials [17]. In another study in patients with localized RCC, pre-treatment blood NLR has been demonstrated as an independent predictor of recurrence [18].

Taken together, the prognostic relevance of neutrophils in localized and metastatic RCC is important and reveals a subgroup of patients with a very poor prognosis and only limited or no effect of cytokine or targeted therapy. Impaired prognostic impact has been noted for baseline blood neutrophils (range 4.5–7.5 × 10^9/L), on-treatment week 5 and week 8 blood neutrophils (range 2.19–4.57 × 10^9/L), and intra-tumoral presence of neutrophils both in localized and metastatic renal cell carcinoma. Further research to unravel the underlying biology is encouraged.

3. Melanoma

The first report of neutrophils as an adverse prognostic factor for patients with metastatic melanoma (MMM) was published in

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