CLINICAL LABORATORY RESULTS EXPRESSED AS RATIOS

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Abstract. It is noticeable that there are many recent studies supporting the use of ratios instead of values of a stand-alone parameter. Elevated neutrophils per lymphocytes ratio (NLR), as a marker of the systemic immune response, has indicated poor prognosis in various cancer types, cancer treatment and cardiovascular diseases. For the late mentioned, NLR has prognostic values as regards short- and long-term mortality in patients with acute coronary syndromes, stable coronary artery disease, heart failure, coronary artery bypass and peripheral arterial disease treated with antiplatelet drugs. Neutrophilia and elevated NLR were associated with decreases in suppressive function of CD4 and CD25 regulatory T cells in acute phases of coronary syndromes. On other occasions, validated, well known ratios like the De Ritis ratio AST per ALT have been modified in order to develop scores with better performances. Among fibrosis risk scores, the AST: platelet ratio index APRI {AST/ULN) x 100}/platelet count (109/L) has been considered more reliable than the AST:ALT ratio. Nevertheless, APRI is used exclusively for selected populations with hepatitis virus C and extending its use to other diagnoses can lead to errors of diagnosis. The De Ritis ratio is now considered questionable as its calculation is requested for patients with transaminases already abnormal. As investigations by now used arbitrary threshold values for the neutrophils to lymphocytes ratio threshold values, future researches are needed to define its cutoffs.
Key words: neutrophils per lymphocytes ratio, AST per platelets ratio index, cardiovascular, cancer

REZULTATELE CLINICE DE LABORATOR EXPRIMATE CA RAPOARTE

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Rezumat. Este lesne de observat că numeroase studii recente sunt în sprijinul utilizării unor rapoarte între două valori numerice, în locul valorilor pentru un singur parametru. Raportul neutrofile per limfocite (NLR) crescut, ca marker al răspunsului inflamator sistemic, indică prognosticul (negativ) în diverse tipuri de cancer, tratamentul cancerului şi boala cardiovasculară. În patologia cardiovasculară, NLR are valori prognostice privind mortalitatea pe termen scurt şi lung în boala coronariană acută, boala coronariană stabilă, insuficienţa cardiacă, bypass aorto-coronarian şi boala arterială periferică sub tratament cu anti-agregante. În boala coronariană acută, neutrofilia şi NLR crescut au fost asociate cu scăderea numărului limfocitelor T-reglatorii, subseturile CD4 şi CD25. Alteori, rapoarte numerice validate şi foarte cunoscute, cum este raportul De Ritis AST:ALT, au fost modificate cu scopul de a introduce scoruri mai performante. Dintre scorurile care indică fibroza ficatului, raportul index AST per plachete APRI{AST/ULN) x 100}/număr trombocite (109/L) este mai sigur comparativ cu raportul de Ritis. APRI este folosit însă exclusiv pentru populaţii selectate cu hepatită virus C, iar extinderea utilizării lui pentru alte diagnostice poate conduce la erori de diagnosticare. În prezent raportul de Ritis este considerat discutabil deoarece calculul este solicitat pentru pacienţi când valorile transaminazelor sunt deja

anormale. Deoarece investigațiile de până acum au utilizat valori prag arbitrare pentru raportul neutrofile per limfocite, noi cercetări sunt necesare pentru a defini valorile limită ale raportului.
Cuvinte cheie: raport neutrofile per limfocite, raportul index AST per număr trombocite, cardiovascular, cancer

INTRODUCTION

It is noticeable that there are by now many clinical researches supporting the use of ratios instead of data of stand-alone parameters. Also, long-time known ratios like the validated De Ritis ratio (AST/ALT) have been modified in order to develop scores with better performances. Future investigations will clarify whether or not to rely on and use these ratios to replace measurements of some parameters of the clinical laboratory.

Of these ratios that of neutrophil to lymphocyte (NLR) emerged as a marker of poor prognosis in cancer and cardiovascular diseases. "More recently, NLR was considered to be a reliable predictor of poor outcomes in clinical hepatic entities". For liver failure from acute and chronic hepatitis B the ratio served as a predictor of short-term mortality. NLR was as well underlined as a simple and easily accessible variable for evaluating therapeutic response in patients with chronic hepatitis C. Nevertheless, the utility of NLR in autoimmune liver disease is still being investigated [1].

PROGNOSTIC VALUE. SENSITIVITY AND SPECIFICITY

I. NEUTROPHILS PER LYMPHOCYTES RATIO - NLR

C-reactive protein (CRP), neutrophils to lymphocyte ratio NLR and the Glasgow Prognostic Score GPS combining CRP and albumin were all reported as indicators of poor prognosis especially in cancer. Tumor-induced systemic inflammatory responses change neutrophils, lymphocytes and platelets levels in peripheral venous blood and contribute to cancer progression and metastasis. Therefore, quantifications of some hematological parameters have been analyzed in various malignant tumors [2-4]. Also, NLR has been used in oncology for monitoring effects of treatments and then its use extended to cardiology. Additionally, increased concentrations of pro-inflammatory cytokines eventually causing cellular DNA damage were reported together with elevated NLR [5,6].

Neutrophils to lymphocyte ratio have been mostly analyzed in studies which investigated pancreatic cancer. According to Gao et al. [2], cancer-associated inflammation is characterized by key molecular features such as inflammatory cytokines, growth factors and proteinases "that are favorable for proliferation, invasion and metastasis of pancreatic cancer cells". Zhou et al. [7] as well as Inoue et al. [8] revealed NLR and CRP prognostic values in large pancreatic cancer cohorts. Low NLR was a favorable predictor of overall survival and disease-free survival DFS in patients with pancreatic cancer [7]. The NLR associations found out in patients with pancreatic cancer were with CA-199 marker, tumor size and cancer stage. Another study on associations of NLR and transition phenotypes of circulating tumor cells CTC showed that the ratio associated with CTC and pointed out that in patients lacking circulating tumor cells, lymphocyte counts and the neutrophil-to-lymphocyte ratio (NLR) were significantly different from those in patients testing positive for CTC subpopulation ($P<0.001$) and circulating tumor microemboli CTMs [9].

It was presumed that markers of the systemic inflammatory response (NLR, CRP) suggest poor prognosis also in cardiovascular diseases. In this sense, Bennites et al. [10] showed that NLR is a better "predictor of mortality than absolute neutrophil and lymphocyte counts in patients with acute decompensated heart failure". A high NLR might be a risk marker for advanced heart failure more than the altered leukocyte composition is.

Several studies documented on lymphocytopenia as frequently predicting poor prognosis for patients with heart failure [11-13]. A study conducted at cellular level [14], using blood samples from patients with heart failure showed that sympathetic activation and oxidative stress/pro-inflammatory statuses activated programmed lymphocyte death, thus causing lymphocytopenia in these patients [14]. As shown by authors, cutoff values indicating mild lymphocytopenia were between more than 1500 and less 2000 cells per microliter and those pointing at severe lymphocytopenia were less than 1500 cells per microliter. In patients who had only heart failure but not lymphocytopenia, lymphocytes were higher than 2000 cells per microliter [14]. Battin et al. [13] pointed out for hospitalized patients with heart failure and protein-losing enteropathy that lymphocytopenia was associated with loss of albumin and concomitant loss of lymphocytes.

Bhat et al. [15] listed as well, cardiovascular diseases for which NLR has prognostic values that indicate poor outcome. The ratio was reported "as an independent predictor of outcome in stable coronary artery disease and a predictor of short- and long-term mortality in patients with acute coronary syndromes, in patients with advanced heart failure and as well a prognostic marker for outcome from coronary artery bypass grafting. In addition, NLR pointed to increased risk of ventricular arrhythmias during percutaneous coronary intervention (PCI).

Spark et al. [16] demonstrated in his study that an elevated NLR predicted high mortality in patients aged 70 years old with peripheral arterial disease and critical limb ischemia who were on antiplatelet therapy. Because of an extremely high mortality rate of 43% in these patients during the 8.7 months-follow-up, additional parameters for risk stratification were necessary. Of these NLR proved clinically relevant at a cutoff value of 5.25, with an overall accuracy in the dataset of 66.4%. Decreases in both number and suppressive function of CD4 and CD25 regulatory T cells, neutrophilia and elevated NLR, were found out in acute phases of coronary syndromes and suggested presence of "circulating leukocyte-platelet aggregates that facilitate vascular plugging and infarct extension" [16].

An important finding was also that not only in cancer research could NLR aid to stratify patients but also in cardiovascular studies. Based on investigations regarding NLR utility in predicting long-term mortality in patients with non–ST-segment elevation myocardial infarction (NSTEMI), Azab et al. [6] showed that patients in the highest NLR tertile (NLR higher than 4.7) had a higher 4- year mortality rate (29.8% vs 8.4%) compared to those in the lowest tertile (NLR less than3). They underscored with regard to all above studies that several of these managed to categorize patients according to NLR intervals (tertiles, quartiles, quintiles), while other studies managed to establish NLR cutoff values [5].

Among limitations of these researches, Lin et al. [1] mentioned underlying mechanisms of elevated NLR that should be explored as the ratio is "a combined parameter of inflammation and host immune surveillance". Therefore, expanded neutrophils eventually give rise to a supporting milieu that enhances tumor growth and invasion but this inflammatory microenvironment is related to alterations in peripheral blood cells including lymphocytes. According to Lin et al. [1] several studies on primary biliary cirrhosis pointed to 10 to 100-fold increases in frequency of auto-reactive intrahepatic differentiation of CD4+ or CD8+ T cells, in comparison to lymphocytes in peripheral blood. Also, contradictory results of lymphocyte counts could be due to the absolute numbers of individual cell types, which change under infection, medication and stress. Azab et al. [5] added another limitation, which is lack of

standardization due to different time-points for collecting and analyzing blood samples.

II. AST: PLATELET RATIO INDEX - APRI

Liver fibrosis is another example illustrating advantages of using ratios as scoring to identify patients who need further investigations of liver disease. Among fibrosis risk scores, the AST: platelet ratio index APRI has been considered more reliable than the AST:ALT ratio as there has been confusion about clear indications of the later [17]. For example, alcohol use raises AST and implicitly the ratio AST: ALT but the latter ALT indicates more advanced liver fibrosis in patients with hepatitis C infection than liver fibrosis of those who abuse alcohol. Therefore, specifications on sensitivity and specificity of the AST:ALT ratio in cases different as regards their primary pathology would be needed. Also, Botros et al. [18] pointed out that a cutoff value for this ratio is questionable since the ratio is calculated for patients with transaminases already abnormal.

Cheung et al. [19] utilized APRI {AST/ULN) x 100}/platelet count (109/L) as a variable predictive of development of hepato-cellular carcinoma HCC in 144 patients with primary biliary cirrhosis. "The overall 5-, 10- and 15-year cumulative incidences of HCC were 2.3% (95% CI: 0% - 4.8%), 8.4% (95% CI: 1.8 - 14.5%) and 21.6% (6.8% - 34.1%), respectively". The study pointed out that APRI higher than 0.54 at one year after treatment (HR=3.94), older age (HR =1.07) and cirrhosis (HR = 4.38) were independent prognostic factors for development of hepato-cellular carcinoma. [19].

Joshita et al. [20] specified that APRI at values higher than 0.54 for patients with primary biliary cirrhosis (n=272) predicted disease progression to hepatic failure defined as decompensated liver cirrhosis in patients with jaundice, ascites, gastrointestinal bleeding, hepato-renal syndrome and encephalopathy. "The 5-, 10-, and 20-year cumulative incidences of hepatic failure were 3.5%, 5.8%, and 12.7%, respectively". Also according to this research, APRI had a high prognostic accuracy (AUROC 0.789, 95% confidence interval: 0.742–0.835) [20].

However, APRI is validated in referral populations with hepatitis C virus and thus, extending it to other conditions or populations may be misleading [21]. Drees et al. [22] lately demonstrated that stand-alone AST was as effective as FIB-4 index and APRI at predicting fibrosis of hepatitis viruses B and C. Also, authors specified that "cutoffs developed for AST, FIB-4 index, and APRI all had specificities of 79.2% to 80.3% for ruling-in severe fibrosis and enabled triage of one third of the study-population". Another advantage of using these indicators was that there was no need to wait for liver biopsies results before starting treatment. Nevertheless, at present there is no single serum fibrosis marker sufficiently sensitive to rule-out significant fibrosis [22].

CONCLUSION

As investigations by now used arbitrary threshold values for the neutrophils to lymphocytes ratio, future researches are needed to define its cutoffs.

Conflicts of interest

The authors declare no conflicts of interest.

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