Neutrophil-Lymphocyte Ratio as an Independent Predictor of Survival in Pulmonary Arterial Hypertension: An Exploratory Study

Naomie Jutras-Beaudoin, BSc, Victoria Toro, MSc, Annie Christine Lajoie, MD, Sandra Breuils-Bonnet, MSc, Roxane Paulin, PhD, and François Potus, PhD
Centre de Recherche de l’Institut Universitaire de Cardiologie et de Pneumologie de Québec (CRIUCPQ), Québec, Québec, Canada

ABSTRACT

Background: The blood neutrophil-to-lymphocyte ratio (NLR) has recently emerged as a powerful predictor of adverse outcomes in some cardiovascular and lung diseases. Pulmonary arterial hypertension (PAH) is a lethal vasculopathy associated with increased inflammation. Although PAH exhibits a higher prevalence among women, men have a poorer prognosis. We investigated the NLR as an independent predictor of transplant-free survival in PAH.

Methods: We performed a retrospective analysis of 78 PAH patients from the Quebec PAHbiobank (71% female). We used univariate and multivariate (adjusted for age, sex, renal function, and disease severity) analyses to determine a threshold that can adequately identify a subset of PAH patients with a high risk of death. We also evaluated the NLR as an independent predictor of transplant-free survival in PAH.

Results: The NLR predicted event-free survival in PAH patients. Interestingly, the NLR was higher in men, with a female-to-male ratio of 4:1. Although the incidence of PAH is higher in women, the prognosis is worse for men. In the Registry to Evaluate Early and Long-term Pulmonary Arterial Hypertension Disease Management (REVEAL) registry, the estimated 5-year survival rate from diagnosis was 52% for men, compared to 62% for women. Surprisingly, this aptly named "sex paradox." Among other factors, the NLR was shown to predict survival in only female patients. Our exploratory work aimed to assess the role of the NLR as an independent predictor of transplant-free survival in PAH and to determine a threshold that can adequately identify a subset of patients with a high risk of death.

Conclusion: The NLR is a promising predictor of transplant-free survival in PAH patients, especially in women. Further studies are needed to confirm its utility in a clinical setting.

RéSUMÉ

Contexte : Le rapport neutrophiles/lymphocytes (RNL) s’est récemment imposé comme un puissant facteur prédictif de l’issue défavorable de certaines maladies cardiovasculaires et pulmonaires. L’hypertension artérielle pulmonaire (HTAP) est une vasculopathie mortelle associée à une inflammation accrue. Bien que sa prévalence soit plus élevée chez les femmes, leur pronostic est plus défavorable chez les hommes. Nous avons étudié le RNL en tant que prédicteur indépendant de la survie sans greffe chez des patients atteints d’HTAP.

Méthodologie : Nous avons effectué une analyse rétrospective du matériau sur l’HTAP de la Biobanque du Québec se rapportant à 78 patients. Nous avons utilisé des analyses univariée et multivariée (ajustées pour l’âge, le sexe, la fonction rénale, et l’état de la maladie) afin de déterminer un seuil qui peut adéquatement identifier un sous-groupe de patients PAH à haut risque de mort.

Résultats : Le RNL a prédit la survie sans événement en PAH. Notamment, le RNL était plus élevé chez les hommes, avec un ratio femme-homme de 4:1. Bien que la prévalence de PAH soit plus élevée chez les femmes, le pronostic est moins favorable pour les hommes. Dans le registre REVEAL, l’estimation de la survie à 5 ans à partir du diagnostic était de 52% chez les hommes, comparée à 62% chez les femmes. Surprenant, cet appelé "paradoxe de sexe," chez les femmes plus susceptibles de souffrir de PAH, mais les hommes ayant une mortalité plus élevée, reste inexpliqué et est rarement considéré dans les études investiguant des marqueurs de PAH. Intéressant à noter est que dans d’autres domaines tels que l’oncologie, le RNL a montré la capacité à prédire la survie dans seulement des femmes. Notre travail exploratoire a visé à évaluer le rôle du RNL comme un prédicteur indépendant de la survie sans greffe en PAH et à déterminer un seuil qui peut adéquatement identifier une sous-population de patients PAH à haut risque de mort.

Conclusion : Le RNL est un prédicteur prometteur de la survie sans greffe en PAH, en particulier chez les femmes. Des études supplémentaires sont nécessaires pour confirmer l’utilité clinique de ce facteur.

Received for publication September 24, 2021. Accepted November 25, 2021.

Ethics Statement: Blood samples were drawn during the right heart catheterization procedure after written informed consent was obtained and approval was received from the Laval University and the Institut Universitaire de Cardiologie et de Pneumologie de Québec (CRIUCPQ) Biobank Ethics Committee (CER #20735).

Corresponding author: Dr. François Potus, 2725, chemin Sainte-Foy, Québec, Québec G1V 4G5, Canada.
E-mail: francois.potus@criucpq.ulaval.ca
See page 362 for disclosure information.
subgroup of patients at higher risk of adverse events. We also aimed to evaluate how sex modulates the association between the NLR and transplant-free survival.

Methods

The Quebec PAH Biobank cohort includes PAH patients from the Quebec Heart and Lung Institute who underwent right heart catheterization (RHC) for suspected pulmonary hypertension or follow-up. PAH was diagnosed according to the current guidelines.1 Blood samples were drawn during the RHC procedure after written informed consent was obtained, and approval was received from the Laval University and l’Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ) Biosafety and Ethics Committees (CER#20735). Blood samples included a whole blood count (CER#20735). Blood samples included a whole blood count and differential blood count (absolute count for neutrophils, lymphocytes), as well as biochemistry analysis (levels of urea, creatinine, and N-terminal of the prohormone brain natriuretic peptide [NT-proBNP]). The NLR was calculated using the absolute neutrophil and lymphocyte counts, whereas the estimated glomerular filtration rate (eGFR) was determined using the modification of diet in renal disease equation (ml/min per 1.73 m²).13 Demographics, medical history, pulmonary function tests, and markers of disease severity (functional class, exercise capacity, pulmonary hemodynamics) were collected contemporarily, whereas incidences of morbidity and mortality events were collected prospectively.

Statistical analysis

All statistical analyses were conducted using the R statistical package (http://www.rproject.org, version 4.0.5), with P < 0.05 considered statistically significant. Continuous variables were reported as means ± standard deviations (SDs), and categorical variables were reported as frequencies (percentages). Between-group comparisons (men vs women and high vs low NLR) were made using either the unpaired t test (parametric), the Mann-Whitney U test (nonparametric), or the χ² test, as applicable.

Univariate Cox regression models were used to identify factors associated with transplant-free survival, using the “survival” and “survminer” R packages. The date of initiation of RHC was the starting point to determine follow-up duration. The cut-off date was May 20, 2021. Patients lost to follow-up were censored as of the date of the last medical visit. Covariates of clinical significance and with P values < 0.05 in the univariate models and n > 70 observations were included in the multivariate Cox regression model. Collinearity was assessed using a correlation matrix (Pearson or Spearman, according to the distribution). Due to the limited number of events, we generated 6 regression models considering clinical variables (age, sex, and eGFR) and one variable reflecting disease severity (NT-proBNP, mixed venous oxygen saturation, right atrial pressure, cardiac index, world health organization [WHO] functional class, and PAH type, respectively, in models 1, 2, 3, 4, 5, and 6). The Kaplan-Meier method was also used to estimate the transplantation-free survival for patients with high (≥ 4.8) or low (< 4.8) NLR, defined as the value with the highest sum of sensitivity and specificity to predict outcomes following receiver operating characteristic (ROC) curve analysis (Wilson/Brown method to a 95% confidence interval). Survival distributions were compared using the log-rank (Mantel-Cox) test and the Gehan-Breslow-Wilcoxon test.

Results

The characteristics of the study population are presented in Table 1. Most patients were WHO functional class III IPAH. During a median follow-up period of 1154 days (range:
Table 1. Baseline characteristic of the Quebec Pulmonary Arterial Hypertension (PAH) Biobank cohort

| Variable                          | PAH (n = 78) | Low NLR < 4.8 (n = 55) | High NLR > 4.8 (n = 23) |
|-----------------------------------|-------------|------------------------|-------------------------|
| Age, y (no. missing)              | 66 ± 17 (0) | 65 ± 17 (0)            | 70 ± 12 (0)*            |
| BMI, kg/m² (no. missing)          | 28± 7 (0)  | 27 ± 8 (0)             | 28 ± 6 (0)              |
| Male/female                       | 26 (33.3)/52.0 (66.7) | 18 (32.7)/57 (67.3) | 8 (34.8)/15 (65.2)     |
| Race                              |             |                        |                         |
| Caucasian                         | 77.0 (98.7) | 54 (98.2)              | 23 (100)                |
| African                           | 1.0 (1.3)  | 1 (1.8)                | 0 (0)                   |
| PAH type                          |             |                        |                         |
| IPAH                              | 50.0 (64.1) | 39 (70.9)              | 11 (47.8)               |
| APAH                              | 25.0 (32.0) | 15 (27.3)              | 10 (43.6)               |
| SSc-PAH                           | 2.0 (2.6)  | 1 (1.8)                | 1 (4.3)                 |
| Heritable PAH                     | 1.0 (1.3)  | 0 (0)                  | 1 (4.3)                 |
| Treatment                         |             |                        |                         |
| ERA                               | 15 (19.2)  | 12 (21.8)              | 3 (13.0)                |
| PDE5 inhibitor                    | 25 (32.0)  | 16 (29.1)              | 9 (39.1)                |
| Prostacyclin                      | 4 (5.1)    | 2 (3.6)                | 2 (8.6)                 |
| Calcium blocker                   | 24 (30.8)  | 18 (32.7)              | 6 (26.1)                |
| Non-treated                       | 29 (37.2)  | 21 (38.2)              | 8 (34.8)                |
| WHO functional class              |             |                        |                         |
| I                                 | 0 (0)       | 0 (0)                  | 0 (0)                   |
| II                                | 16.0 (20.5) | 12 (21.9)              | 4 (17.4)                |
| III                               | 49.0 (62.8) | 35 (63.6)              | 14 (60.9)               |
| IV                                | 11.0 (14.1) | 6 (10.9)               | 5 (21.7)                |
| II or III                         | 1.0 (1.3)  | 1 (1.8)                | 0 (0)                   |
| Unknown                           | 1.0 (1.3)  | 1 (1.8)                | 0 (0)                   |
| 6MWD, m (no. missing)             | 323 ± 171(13) | 341 ± 169 (6) | 272 ± 170 (7)*        |
| Hemodynamic parameters (no. missing) |         |                        |                         |
| Mean PAP, mm Hg                   | 47 ± 14 (1) | 48 ± 15 (1)            | 44 ± 14 (0)             |
| RAP, mm Hg                        | 7.0 ± 7.0 (1) | 7.0 ± 6.0 (1) | 10.5 ± 6.2 (0)*       |
| CI, L/min per m²                  | 2.4 ± 1.1 (1) | 2.4 ± 1.0 (1) | 2.1 ± 1.2 (0)*        |
| PVRI, dynes/cm² per m²            | 1271 ± 846 (2) | 1248 ± 866 (1) | 1412 ± 984 (0)        |
| PCWP, mm Hg                       | 9.0 ± 5.0 (2) | 9.0 ± 4.8 (1) | 8.0 ± 7.0 (0)         |
| SVo₂, %                           | 65 ± 14 (1) | 66 ± 10 (2)            | 56 ± 13 (1)            |
| TAPSE, mm                         | 18.0 ± 14 (57) | 20.0 ± 10.0 (44) | 14.0 ± 8.2 (13)*      |
| Blood count, % (no. missing)      |             |                        |                         |
| WBC, ×10⁹ cells/L                | 8.4 ± 3.0 (1) | 7.9 ± 3.1 (0) | 9.3 ± 3.5 (1)         |
| ALC, ×10⁹ cells/L                | 1.5 ± 1.1 (1) | 1.8 ± 0.8 (0) | 0.9 ± 0.5 (1)         |
| ANC, ×10⁹ cells/L                | 5.8 ± 2.6 (1) | 5.1 ± 2.1 (1) | 7.5 ± 3.6 (1)         |
| NLR                               | 3.3 ± 2.5 (1) | 3.0 ± 1.2 (0) | 8.1 ± 5.2 (1)         |
| Biochemistry                      |             |                        |                         |
| NT-ProBNP, pg/ml (no. missing)    | 1207 ± 2666 (0) | 725 ± 2078 (1) | 2602 ± 5840 (0)*     |
| eGFR, mL/min per 1.73m² (no. missing) | 68 ± 32 (0) | 72 ± 33 (0) | 61 ± 28 (0)*         |

Values are n (%), or median ± interquartile range, unless otherwise specified. *P values determined by unpaired t test, Mann Whitney, or χ² test.

ALC, absolute lymphocytes count; ANC, absolute neutrophil count; APAH, associated PAH; BMI, body mass index; CI, cardiac index; eGFR, estimated glomerular filtration rate; ERA, endothelin receptor antagonist; IPAH, idiopathic PAH; NLR, neutrophil-to-lymphocyte ratio; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; PAH, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PDE5, phosphodiesterase 5; PVRI, pulmonary vascular resistance index; RAP, right atrial pressure; SSc-PAH, scleroderma-associated PAH; SVO₂, central mixed venous oxygen saturation; TAPSE, tricuspid annular plane systolic excursion; WBC, white blood cell count; WHO, World Health Organization. 6MWD, 6-minute walk distance.

*P < 0.05.  †P < 0.0001.  ‡P < 0.01.

7-3170 days), 41 PAH patients died (n = 39) or were referred for transplantation (n = 2). The NLR was significantly higher in patients who died or were referred for transplantation (P = 0.0319; Fig. 1A). The ROC curve analysis suggested 4.8 as the most discriminative value to predict poor outcomes (area under the curve: 0.64; P = 0.03; Supplemental Fig. S1). Patients in the high-NLR group were older, and had increased disease severity and decreased lung function compared to those in the low-NLR group (Table 1). The estimated 1-, 3-, and 5-year transplant-free survival rates were 65.2%, 21.3-4%, and 10.6%, respectively, in the high-NLR group, compared with 86.8%, 72.7% and 57.6% in the low-NLR group, respectively (Fig. 1B). This association was confirmed using univariate Cox regression models, which showed that the NLR was associated with an increased risk of death/transplant referral (hazard ratio [HR]: 1:11; 95% confidence interval [CI]: 1:04-1:18; Table 2). In multivariate analyses, the NLR remained independently associated with an increased risk of mortality/transplantation after adjusting for age, sex, eGFR, and disease severity, as defined by the NT-proBNP level (model 1; HR: 1:12; 95% CI: 1:04-1:21), SvO₂ level (model 2; HR: 1:17; 95% CI: 1:08-1:27), right atrial pressure (model 3; HR: 1:13; 95% CI: 1:04-1:23), cardiac index (model 4; HR: 1:15; 95% CI: 1:06-1:24), and
We investigated whether the PAH subtype affects the NLR. We observed that NLR is significantly higher in scleroderma-associated PAH patients, compared with IPAH patients \((P = 0.0135; \text{Supplemental Fig. S2A})\), and can be used to successfully discriminate between these 2 PAH subtypes (area under the curve \(= 0.679; P = 0.0142; \text{Supplemental Fig. S2B}\)). Both IPAH and CTD-PAH patients with NLR \(> 4.8\) exhibit lower survival, compared with patients with NLR \(< 4.8\) (Supplemental Fig. S2C and D).

Surprising to note is that PAH subtype was not associated with transplant-free survival (univariate analysis \(HR = 1.22; 95\% \text{ CI: } 0.89-1.67; P = 0.206; \text{Table 1}\)), and NLR remains an independent predictor of survival when corrected for PAH type (model 6; Suplemental Table S1).

Lastly, we explored whether a sexual dimorphism was observed in PAH. Men and women had similar disease severity and NLR (Supplemental Table S2). An intriguing point is that the NLR was significantly higher in female PAH patients who died \((7.0 \pm 5.6, \text{ vs } 2.9 \pm 0.9; P = 0.0386)\) but not in male patients \((4.2 \pm 1.3, \text{ vs } 4.3 \pm 1.9; P = 0.8425; \text{Fig. 2A; Supplemental Fig. S3A})\). Consistently, the NLR predicted transplantation-free survival in women only, with an estimated 1-, 3-, and 5-year transplant-free survival rate of 73.3%, 38.9%, and 15.5%, respectively, compared with 97.2%, 88.0%, and 73.9% in the high- and low-NLR groups, (Fig. 2B). We observed no evidence of an association between the NLR and survival in men (Supplemental Fig. S3B).

### Table 2. Cox regression models

| Variable               | HR     | 95% CI     | \(P\) | Sample size |
|------------------------|--------|------------|-------|-------------|
| Age                    | 1.05   | 1.02–1.09  | <0.001| 78          |
| Sex (male)             | 2.61   | 1.41–4.83  | 0.002 | 78          |
| BMI                    | 1.01   | 0.96–1.06  | 0.683 | 78          |
| PAH type               | 1.20   | 0.89–1.64  | 0.235 | 78          |
| WHO functional class   | 2.79   | 1.45–5.39  | 0.002 | 77          |
| 6MWD, m                | 0.99   | 0.99–1.00  | 0.003 | 65          |
| Hemodynamic parameters |         |            |       |             |
| Mean PAP, mm Hg        | 1.00   | 0.98–1.03  | 0.770 | 77          |
| RAP, mm Hg             | 1.08   | 1.01–1.15  | 0.025 | 77          |
| CI, L/min per m\(^2\) | 0.57   | 0.37–0.87  | 0.010 | 78          |
| PVRI, dynes/cm\(^5\) per m\(^2\) | 1.01 | 0.99–1.01 | 0.058 | 76         |
| PCWP, mm Hg            | 1.02   | 0.92–1.14  | 0.674 | 77          |
| SvO\(_2\), %           | 0.92   | 0.88–0.96  | <0.001| 75          |
| Blood counts           |         |            |       |             |
| WBC \((\times 10^9)\) cells/L | 1.17 | 1.01–1.35 | 0.032 | 77          |
| ANC \((\times 10^9)\) cells/L | 1.22 | 1.06–1.41 | 0.006 | 77          |
| ALC \((\times 10^9)\) cells/L | 0.51 | 0.29–0.88 | 0.016 | 77          |
| NLR                    | 1.11   | 1.04–1.18  | 0.002 | 77          |
| Biochemistry           |         |            |       |             |
| eGFR, mL/min per 1.73 m\(^2\) | 0.98 | 0.96–0.99 | 0.001 | 78          |
| NT-proBNP (pg/ml) \(^\text{1}\) | 1.01 | 1.00–1.01 | 0.009 | 77          |

**Note:** For each 10 units of PVRI.

**Note:** For 100 units of NT-proBNP.

### Discussion

In this retrospective analysis of the Quebec PAH-biobank cohort, we observed that the NLR was a successful and independent predictor of transplant-free survival in PAH patients. We also identified an NLR threshold \((\geq 4.8 \text{ vs } < 4.8)\) that categorizes a subgroup of PAH patients with higher disease severity and higher risk of death/transplant. The association between the NLR and transplant-free survival in PAH appears to be greater for women, compared to men.
thereby suggesting a potential sexual dimorphism in the ability of the NLR to predict transplant-free survival in PAH. However, the strength of this finding may be mitigated by the small number of men available for the analysis. Furthermore, we also report that an increased NLR can discriminate between patients with scleroderma-associated PAH vs IPAH. The NLR has been shown to predict mortality in various populations. In retrospective studies involving patients with various malignancies (breast, colorectal, esophageal, liver, melanoma, ovarian, pancreatic, prostate), and with lung diseases (eg, chronic obstructive pulmonary disease), the NLR successfully predicted survival. Furthermore, population-based studies demonstrated that an increased NLR was an independent predictor of heart failure and cardiovascular mortality. Interesting to note is that the NLR was an independent predictor of right-ventricular dysfunction in patients with inferior ST-segment elevation myocardial infarction. We observed that PAH patients with a high NLR had a lower tricuspid annular plane systolic excursion (Table 1). These findings suggest that a high NLR could reflect the propensity for right-ventricular dysfunction, which is of particular relevance in PAH, in which right-ventricular failure remains the main cause of death. However, such hypotheses remain speculative, and further studies looking at the relationship between NLR and right-ventricular function are needed.

Our findings supporting the role of the NLR as a predictor of event-free survival in PAH are in accordance with those of previous work. In an exploratory cohort study of 77 PAH patients, Harbaum et al. also concluded that an increased NLR was an independent predictor of death or transplantation. However, these results contrast with those of other studies that failed to identify NLR as an independent predictor of death in PAH patients, despite its ability to adequately discriminate PAH patients from healthy controls (NLR = 2.44 in PAH patients vs 1.55 in controls). Nonetheless, the average NLR value observed in their cohorts was substantially lower than the one reported in our study (2.44 vs 4.90). Normal NLR values in a non-geriatric and otherwise healthy adult population range between 0.78 and 3.53. Thus, an NLR above the upper limit of the normal range (> 3.5) most likely reflects an abnormal state of increased systemic inflammation and physiological stress. Furthermore, age, sex, and race have also been known to influence the NLR. Genetic ancestry could thus explain these differences, as studies reporting a higher NLR (>3.5) included mostly Caucasians from Canada and Germany, whereas those with lower NLR values originated from Turkey. Thus, discrepancies in the reported NLR values for PAH patients may stem from underlying patient characteristics.

Although NLR is moderately increased in transplanted or deceased patient groups (Fig. 1A; Supplemental Fig. S1), patients with a high NLR (NLR < 4.8) exhibit a significantly lower survival rate on a median follow-up period of 1154 days. The NLR threshold identified in our study (4.8) is strikingly similar to the NLR threshold previously reported by

### Multivariate Cox regression models

| Model 1 (NT-proBNP) | HR | 95% CI | P  | Sample size |
|---------------------|----|--------|----|-------------|
| Model 2 (SvO2)     | 1.12 | 1.04–1.21 | 0.002 | 76 |
| Model 3 (RAP)      | 1.17 | 1.08–1.27 | < 0.001 | 74 |
| Model 4 (Cardiac Index) | 1.13 | 1.04–1.23 | 0.003 | 76 |
| Model 5 (WHO functional class) | 1.15 | 1.06–1.24 | < 0.001 | 76 |

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; RAP, right atrial pressure; SvO2, central mixed venous oxygen saturation; WHO, World Health Organization.

*All models were adjusted for age, sex, and estimated glomerular filtration rate. Additional variables for which the models were adjusted are indicated in parentheses.

---

**Figure 2.** Sexual dimorphism and the ability of the neutrophil-to-lymphocyte (NLR) ratio to predict mortality in patients with pulmonary arterial hypertension. (A) The NLR in blood of deceased or transplanted patients (n = 20), compared with that in alive female patients with pulmonary arterial hypertension (n = 32). (B) Survival curve in female patients with low NLR (< 4.8) and high NLR (≥ 4.8). Comparison between survival curve, log-rank (Mantel-Cox) test, and Gehan-Breslow-Wilcoxon test. ****P < 0.0001.
Harbaum et al. (> 4.14), using a similar approach based on an ROC curve analysis. However, Harbaum et al. reported a lower level of circulating neutrophils and lymphocytes in their PAH sample (absolute neutrophil count: 5.46 × 10^9/L, vs 6.12 × 10^9/L in our study; absolute lymphocyte count: 1.55 × 10^9/L, vs 1.6 × 10^9/L in our study). This discrepancy could be explained by technical factors related to the timing of the collection of blood samples. In the Harbaum et al. study, they collected their samples at least 1 week before RHC, whereas in our study, samples were collected during RHC. Invasive procedures are well known to induce stress and modify the level of circulating white blood cells, particularly neutrophils (also called neutrophil demargination).

Despite these differences in levels of circulating neutrophils and lymphocytes, the reported NLRs in our study were similar (average NLR value = 4.9 in both PAH cohorts), suggesting that it is a reproducible measurement regardless of timing and sample-collection methods.

A well described sex paradox occurs in PAH—the prevalence of the disease is substantially higher in women, compared with that in men, but men have a worse prognosis. Corroborating this observation, women seem to be more resistant to right-ventricular dysfunction and failure. According to the literature, male sex is associated with a significantly greater risk of mortality/transplant referral (HR: 2.6). We observed that deceased female PAH patients had a higher NLR, compared with that of female PAH survivors. However, despite a similar baseline NLR and disease severity, male survivor vs deceased PAH patients exhibit no NLR difference. Furthermore, men with an NLR < 4.8 exhibit a low survival rate, compared with that of women (1- and 5-year transplant-free survival rates of 72.2% and 29.6%, respectively, in men, compared with 94.7% and 89.1% in women). This finding suggests that underlying sex-related differences may affect the ability of the NLR to predict event-free survival in PAH patients. This observation is in line with previous observations made in cancer patients. In a cohort study of 1222 patients with head and neck squamous cell carcinoma, no significant differences were seen in baseline NLR between male and female patients. After patients were categorized according to NLR quartiles, the NLR successfully predicted mortality, with patients in the upper quartile having lower survival. However, when the sample was further categorized according to sex, the NLR predicted survival in only female patients.

Jung and colleagues showed that NLR is associated with connective tissue diseases. They observed an increased NLR in patients with scleroderma, compared with that in control patients, and reported that patients with scleroderma and interstitial lung disease had a higher NLR than those without interstitial lung disease. As expected, we observed that NLR is increased in patients with an immunologic etiology for PAH, such as CTD (scleroderma), compared with that in IPAH patients. However, in our cohort, PAH type is not associated with survival, and the ability of the NLR to predict survival is independent of PAH type. This observation might be a consequence of the low survival rate observed in our cohort. For example, we observed a 5-year survival rate of 43% in our cohort, compared to 61% in the REVEAL cohort. This high mortality rate might be explained by the higher age of the patients in our cohort (62.7 years, vs 51.7 years in the REVEAL cohort). Indeed, in the REVEAL cohort, the 5-year survival decreased as PAH patients aged (70.7%, 52.4%, 42.8%, respectively, in patients aged 35-54, 55-64, and > 64 years). Furthermore, patients enrolled in our cohort exhibited relatively severe disease, with 77% of the patients being in WHO functional class III-IV (vs 53% of patients in WHO class III-IV in the REVEAL cohort).

Although inflammation is a well established determinant of PAH development and progression, no inflammatory-based biomarker is available for the clinical management of PAH. Use of the NLR would allow integration of inflammation into PAH risk stratification. Biomarkers used in the clinic for risk stratification and outcome prediction include clinical signs of right heart failure, 6-minute walk test distance, cardiopulmonary exercise testing, imaging (echocardiography, cardiac magnetic resonance), hemodynamics (right heart catheterization), and NT-proBNP levels, which are not readily available in nonspecialized centres. The NLR has the advantages of being easily available from complete blood count as well as cost-efficient. Finally, the NLR might represent the first inflammatory-based biomarker for prediction of clinical outcome in PAH.

Limitations

Limitations of our study include the limited sample size, especially with the number of male PAH patients. This low number may have impaired our ability to detect statistically significant associations between the NLR and transplant-free survival in this group. The Quebec PAH Biobank cohort comprises 98.7% Caucasians. This limitation precludes any extrapolation of our findings to other ethnicities (eg, Hispanic). Due to the modest sample size, we did not investigate the NLR level in “heritable” and “other” PAH subtypes. We did not validate our observations in an independent validation cohort.

Conclusions

We demonstrated that the NLR is an independent predictor of event-free survival in PAH, supporting the role of the NLR as a reliable, minimally invasive, and easily accessible biomarker in PAH. Furthermore, we report a sexual dimorphism in the ability of NLR to predict mortality, further underlining the importance of integrating sex as a variable in studies evaluating the development of novel biomarkers in PAH.

Acknowledgements

The authors greatly appreciate the intellectual input from members of the Quebec Heart and Lung Institute Pulmonary Hypertension Research Group.

Funding Sources

F.P. receives grants from the Quebec Heart and Stroke Foundation and the Quebec Respiratory Health Research Network.

Disclosures

The authors have no conflicts of interest to disclose.
References

1. Simonneau G, Montani D, Celemajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J 2019;53. 1801913.

2. Benza RL, Miller DP, Barst RJ, et al. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. Chest 2012;142:448-56.

3. Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary arterial hypertension: pathogenesis and clinical management. BMJ 2018;360:j5492.

4. Hirani N, Brunner NW, Kapasi A, et al. Canadian Cardiovascular Society/Canadian Thoracic Society position statement on pulmonary hypertension. Can J Cardiol 2020;36:977-92.

5. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. Circ Res 2014;115:165-75.

6. Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. The neutrophil-to-lymphocyte ratio in pulmonary arterial hypertension. J Int Med Res 2015;43:661-75.

7. Özpelit E, Akdeniz B, Özpelit ME, et al. Association between neutrophil to lymphocyte ratio and cardiovascular disease risk: a systematic review and meta-analysis. Biomed Res Int 2018;2018. 2705518.

8. Yıldız A, Kaya H, Ertuş F, et al. Association between neutrophil to lymphocyte ratio and pulmonary arterial hypertension. Turk Kardiyol Dern Ars 2013;41:604-9.

9. Harbaum L, Baaske KM, Simon M, et al. Exploratory analysis of the neutrophil to lymphocyte ratio in patients with pulmonary arterial hypertension. BMC Pulm Med 2017;17:72.

10. Mair KM, Johansen AK, Wright AF, Wallace E, MacLean MR. Pulmonary arterial hypertension: basis of sex differences in incidence and treatment response. Br J Pharmacol 2014;171:567-79.

11. Shapiro S, Traiger GL, Turner M, et al. Sex differences in the diagnosis, treatment, and outcome of patients with pulmonary arterial hypertension enrolled in the registry to evaluate early and long-term pulmonary arterial hypertension disease management. Chest 2012;141:363-73.

12. Lin CY, Kwon H, Rangel Rivera GO, et al. Sex differences in using systemic inflammatory markers to prognosticate patients with head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2018;27:1176-85.

13. Zabel JR, Larson G, Koffel J, et al. Use of the modification of diet in renal disease equation for estimating glomerular filtration rate in the urologic literature. J Endourol 2016;30:930-3.

14. Fest J, Ruiter TR, Groot Koerkamp B, et al. The neutrophil-to-lymphocyte ratio is associated with mortality in the general population: The Rotterdam Study. Eur J Epidemiol 2019;34:463-70.

15. Wang Q, Li J, Wang X. The neutrophil-lymphocyte ratio is associated with postoperative mortality of cardiac surgery. J Thorac Dis 2021;13:67-75.

16. Palogiannis P, Fois AG, Sotgia S, et al. Neutrophil to lymphocyte ratio and clinical outcomes in COPD: recent evidence and future perspectives. Eur Respir Rev 2018;27. 170113.

17. Kim S, Eliot M, Koester DC, Wu WC, Kelsey KT. Association of neutrophil-to-lymphocyte ratio with mortality and cardiovascular disease in the Jackson Heart Study and modification by the Duffy antigen variant. JAMA Cardiol 2018;3:455-62.

18. Howard R, Kanetsky PA, Egan KM. Exploring the prognostic value of the neutrophil-to-lymphocyte ratio in cancer. Sci Rep 2019;9:19673.

19. Tan TP, Arekapudi A, Metha J, Prasad A, Venkatraghavan L. Neutrophil-lymphocyte ratio as predictor of mortality and morbidity in cardiovascular surgery: a systematic review. ANZ J Surg 2015;85:414-9.

20. Yaylak B, Ede H, Baykal E, Altuntas B, et al. Neutrophil/lymphocyte ratio is associated with right ventricular dysfunction in patients with acute inferior ST-segment elevation myocardial infarction. Cardiol J 2016;23:100-6.

21. Forget P, Khalifa C, Defour JP, et al. What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res Notes 2017;10:12.

22. Azab B, Camacho-Rivera M, Taioli E. Average values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. PLoS One 2014;9:e112361.

23. Tabuchi Y, Shinka S, Ishida H. The effects of anesthesia and surgery on count and function of neutrophil. J Anesth 1989;3:123-31.

24. Romeo C, Crucetti A, Tuiria E, et al. Monocyte and neutrophil activity after minor surgical stress. J Pediatr Surg 2002;37:741-4.

25. Keen J, Prisco SZ, Prins KW. Sex differences in right ventricular dysfunction: insights from the bench to bedside. Front Physiol 2020;11. 623129.

26. Jung J-H, Lee Y-M, Lee E-G, Yoo W-H, Lee W-S. Neutrophil-to-lymphocyte ratio in diagnosis of systemic sclerosis for prediction of interstitial lung disease. J Respir Dis 2017;24:138-42.

27. Farber HW, Miller DP, Poms AD, et al. Five-year outcomes of patients enrolled in the REVEAL Registry. Chest 2015;148:1043-54.

28. Tabuchi Y, Shinka S, Ishida H. The effects of anesthesia and surgery on count and function of neutrophil. J Anesth 1989;3:123-31.

29. Yao Q, Pados JC, Prins KW. Sex differences in right ventricular dysfunction: insights from the bench to bedside. Front Physiol 2020;11. 623129.

30. Jung J-H, Lee Y-M, Lee E-G, Yoo W-H, Lee W-S. Neutrophil-to-lymphocyte ratio in diagnosis of systemic sclerosis for prediction of interstitial lung disease. J Respir Dis 2017;24:138-42.

31. Farber HW, Miller DP, Poms AD, et al. Five-year outcomes of patients enrolled in the REVEAL Registry. Chest 2015;148:1043-54.

32. Galié N, Channick RN, Frantz RP. Risk stratification and medical therapy of pulmonary arterial hypertension. Eur Respir J 2019;53. 1801889.

Supplementary Material

To access the supplementary material accompanying this article, visit CJC Open at https://www.cjcopen.ca/ and at https://doi.org/10.1016/j.cjco.2021.11.010.