Association between serum low-density neutrophils and acute-onset and recurrent Guillain–Barré syndrome

Kaixi Ren | Aili Yang | Jiarui Lu | Daidi Zhao | Miao Bai | Jiaqi Ding | Tiaoxia Wei | Hongzeng Li | Jun Guo

1 Department of Neurology, Tangdu Hospital, Fourth Military Medical University, Xi’an, China
2 Department of Endocrinology, Tangdu Hospital, Fourth Military Medical University, Xi’an, China
3 State Key Laboratory of Cancer Biology, Department of Medical Genetics and Developmental Biology, Fourth Military Medical University, Xi’an, China

Correspondence
Jun Guo, Department of Neurology, Tangdu Hospital, Fourth Military Medical University, Xi’an, China.
Email: guojun_81@163.com

Kaixi Ren and Aili Yang contributed equally to this work.

Abstract

Background and Aim: Guillain–Barré syndrome (GBS) is one of the most common causes of acute flaccid paralysis. A timely assessment of this disease condition and its treatments are of vital importance to patients diagnosed with GBS. The purpose of this study is to investigate the variation trend of neutrophils along with disease courses and assess the prognostic value of serum low-density neutrophils (LDNs) in the acute-onset and recurrence of GBS.

Methods: A total of 176 GBS patients were recruited. Patients were evaluated with Medical Research Council (MRC) sum score and the Hughes Functional Grading Scale score upon admission. Peripheral blood samples were collected for routine testing. Flow cytometry analysis was performed to identify LDNs. All patients were followed up to collect disease condition data.

Results: The total neutrophil ratios and counts were significantly higher in patients with acute-onset GBS compared to healthy controls (HCs). These counts/ratios decreased during remission and re-elevated in recurrent GBS patients. However, no correlation was observed between the total neutrophil counts/ratios and the MRC sum score. The LDNs collected from different GBS courses were identified using flow cytometry. The counts and ratios were significantly higher in acute-onset GBS and recurrent GBS compared to HCs and patients in remission. The LDN counts/ratios displayed a negative correlation with the MRC sum scores in acute-onset GBS and recurrent GBS.

Conclusion: Our findings suggest that LDN counts/ratios are positively correlated with the acute-onset and recurrence of GBS and its severity. Therefore, LDNs might serve as an accessible prognostic indicator for disease progression monitoring.

Keywords
acute-onset, Guillain–Barré syndrome, low-density neutrophils, prognostic indicator, recurrence
1 | INTRODUCTION

Guillain–Barré syndrome (GBS) is one of the most common causes of acute flaccid paralysis. It is characterized by acute onset with preceding infection, limb weakness, areflexia, and cerebrospinal fluid albuminocytological dissociation (Asbury & Cornblath, 1990; Carmona-Rivera & Kaplan, 2013; Casserly et al., 2017). Timely assessment of this disease condition is of vital importance during the acute phase for treatment decisions and prognostic evaluations. Although GBS is often characterized by self-limiting and monophasic disease courses, recurrent cases are noticed in 5%–10% of patients (Cloke et al., 2012; Coffelt et al., 2015). These patients experience multiple periods of deterioration. However, to date, there is a lack of promising indicators that guide the initiation of treatment for GBS until disease progression. Thus, identifying prognostic factors to determine the phase of acute onset and recurrence of GBS is essential for clinical decisions and disease recovery.

Neutrophils play significant roles in both acute and chronic inflammation by regulating the inflammatory process through cytokine secretion, immune cell recruitment, microbe phagocytosis, and antimicrobial molecule degranulation (Denny et al., 2010). Increasing evidence suggests that neutrophils are heterogeneous in morphology and function (Drifte et al., 2013). Low-density neutrophils (LDNs), initially observed by density gradient isolation, have recently been recognized as a subtype of neutrophils with particular features (Fortunati et al., 2009). It has been reported that LDNs expressing CD15+CD11b+CD33+HLA-DR+ are activated neutrophils that undergo degranulation and a series of inflammatory processes in multiple autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis, and arthritis (Fujimi et al., 2012; Goodfellow & Willison, 2016; Grand'Maison et al., 1992; Grayson et al., 2015). Also, a series of nerve system demyelinating autoimmune diseases, for instance, neuromyelitis optica spectrum disorder (NMOSD), multiple sclerosis (MS), and autoimmune encephalitis have been studied to have relevance to neutrophils (Hacbarth & Kajdacsy-Balla, 1986; Hassani et al., 2020; Hoffmann et al., 2013; Huang et al., 2018). However, the association between LDNs and the acute onset and recurrence of GBS remains unclear.

The present study aimed to analyze the variation trend of neutrophils along with disease courses and assess the prognostic value of LDNs in the acute onset and recurrence of GBS.

2 | METHODS AND METHODS

2.1 | Patients

We examined 187 patients diagnosed with GBS between July 2014 and July 2019 at Tangdu Hospital, China. Clinical evaluations were performed immediately upon admission. Routine blood tests were performed for all inpatients. These patients were diagnosed as classical GBS subtypes based on the National Institute of Neurological Disorders and Stroke criteria (NINCDS) (Hüner et al., 2018), including acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor and sensory axonal neuropathy (AMSAN). Clinical diagnosis was supported by symptoms of acute flaccid paralysis, progressive phase period, nerve conduction velocity tests (Inoue et al., 2003), and cerebrospinal fluid albuminocytological dissociation. The eligibility criteria included age group of >15 years of both gender; patients with progressive weakness in limbs and areflexia in weak limbs. Symptomatic progressions were described as definite neurological aggravation compared with the patient’s condition the day before. Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and variant subtypes of GBS, like Miller–Fisher syndrome patients, were excluded from the present study. Also, we excluded 11 patients with coexisting diseases that might have influenced the evaluation: diabetes (n = 7), confirmed cancer (n = 3), and systemic vasculitis (n = 1). A total of 176 patients were included in the study and evaluated using the Medical Research Council (MRC) sum score and Hughes functional grading scale score (Kieseier et al., 2018). Briefly, the MRC sum score is used to evaluate six groups of muscles including proximal and distal upper limbs and lower limbs on both sides, with scores ranging from 0 to 60. The individual muscle group is scored based on myodynamia from 0 to 5: 0, no visible contraction; 1, visible contraction without movement; 2, active movement of the limb, but not against gravity; 3, active movement against gravity over (almost) the full range; 4, active movement against gravity and resistance; and 5, normal power. Hughes functional grading scale score ranges from 1 to 6: 1, minor symptoms and capable of running; 2, able to walk 10 m or more without assistance but unable to run; 3, able to walk 10 m across an open space with help; 4, bedridden or chairbound; 5, requiring assisted ventilation for at least part of the day; and 6, dead. In addition, age, sex, infection history, sensory deficits, cranial nerve involvement, and acute treatment (intravenous immunoglobulins [IVIgs] or plasma exchanges [PEs]) were recorded. In addition, 144 healthy donors were recruited for the blood tests. This study was approved by the Ethics Committee of Tangdu Hospital (TDLL-KY-202106-02). All patients and healthy donors provided written informed consent to participate in the study.

2.2 | Routine blood tests and flow cytometry

Peripheral blood samples were collected for routine blood tests and identified through flow cytometry during the first hospitalization, clinical remission, and disease recurrence. Briefly, blood was freshly collected into anticoagulant tubes from patients and healthy donors on experimental days. As for flow cytometry analysis, blood was added into red blood cells lysis buffer immediately after collection. After the process of red blood cells lysis, cells were centrifuged to collect the remaining white blood cells for further cell counting and fluorescent-conjugated antibodies staining. All procedures were performed in a dark room. 7-aminoactinomycin D (7AAD) was added in the resuspension solutions before tests to eliminate dead cells. Cells were never
allowed to be frozen or kept overnight before FACS analysis. FITC anti-HLA DR (#307604), Pacific Blue anti-CD11b (#101224), PE-Cy7 CD15 (#323030), and APC-Cy7 CD33 (#366614) were purchased from BioLegend (San Diego, CA, USA). Flow cytometry analysis was carried with an FACSCanto flow cytometer (BD Immunocytometry Systems, San Jose, CA, USA).

2.3 Statistical analyses

Statistical analyses were performed by GraphPad Prism software (7.0 version). Categorical data are presented as numbers and percentages, and quantitative data are presented as the mean ± standard error of the mean. Intergroup differences were assessed by one-way analysis of variance followed by Bonferroni post hoc test, and Pearson’s correlation analysis was performed to examine the association between the MRC sum score and total neutrophil ratios/counts and LDN ratios/counts. *p < .05* was considered statistically significant.

3 RESULTS

3.1 Patient characteristics

We recruited 176 GBS patients who met the diagnostic criteria for AIDP and AMAN. The incidence rate and recurrence rate were similar among all age groups. A total of 83 (47.2%) patients reported having a history of infection. The patients with Hughes functional grading scale score ≥ 4 accounted for 35.8% and 52.9% in acute-onset GBS and recurrent GBS patients, respectively. Sensory deficits were observed in 64 (36.4%) patients. Thirteen (7.39%) of the total patients reported cranial nerve involvement. The proportion of patients who received IVIg or PE was 92.6%. We followed up with all patients for at least 12 months since disease onset. After hospital treatment and discharge, 15 patients reported GBS recurrence within 3 months, and two of them reported two recurrences (Table 1). Due to lack of compliance after improvement or economic reasons, only 51 improved patients returned to the hospital for re-examination as requested.

3.2 Variation of total neutrophils in the whole Guillain–Barré syndrome course

The total neutrophil ratios and counts were acquired by routine blood tests from healthy controls (HCs), GBS patients with acute-onset GBS, GBS patients in remission, and recurrent GBS patients. As shown in Figure 1a, neutrophil ratios (64.6 ± 1.05%) and counts (4.84 ± 0.20 × 10E9/L) were significantly elevated in acute-onset GBS patients compared with HCs (57.1 ± 0.73%, 3.34 ± 0.11 × 10E9/L) and decreased in remission patients along with improved symptoms (60.7 ± 1.49%, 4.02 ± 0.24 × 10E9/L). However, in recurrent GBS patients, neutrophil ratios and counts increased again (65.3 ± 2.64%, 5.48 ± 0.55 × 10E9/L).

### TABLE 1 Characteristics of the patients (n = 176)

| Variables                                               | Acute-onset GBS n (%) | Recurrent GBS n (%) |
|---------------------------------------------------------|-----------------------|---------------------|
| **Total**                                               | 176                   | 17                  |
| **GBS subtypes**                                        |                       |                     |
| AIDP                                                    | 96 (54.5%)            | 9 (52.9%)           |
| Axonal GBS (AMAN/AMSAN)                                 | 65 (37.0%)            | 6 (35.3%)           |
| Equivocal                                               | 15 (8.5%)             | 2 (11.8%)           |
| **Age (years)**                                         |                       |                     |
| > 60                                                    | 35 (19.9%)            | 3 (17.6%)           |
| 41–60                                                   | 116 (65.9%)           | 11 (64.7%)          |
| ≤ 40                                                    | 25 (14.2%)            | 3 (17.7%)           |
| **Female/male**                                         | 104/72 (59.1%/40.9%)  | 12/5 (70.6%/29.4%)  |
| **Symptoms preceding infection**                       |                       |                     |
| Diarrhea                                                | 45 (25.6%)            | 7 (41.2%)           |
| Upper respiratory tract infection                       | 38 (21.6%)            | 10 (58.8%)          |
| **NCV findings**                                        |                       |                     |
| Demyelinated                                           | 96 (54.6%)            | 9 (52.9%)           |
| Axonal                                                  | 65 (37.0%)            | 5 (29.4%)           |
| Equivocal                                               | 10 (5.70%)            | 3 (17.7%)           |
| Normal                                                  | 5 (2.80%)             | 0 (0.00%)           |
| **Hughes functional grading scale score**               |                       |                     |
| 1                                                       | 18 (10.2%)            | 0 (0.00%)           |
| 2                                                       | 32 (18.2%)            | 2 (11.8%)           |
| 3                                                       | 63 (35.8%)            | 6 (35.3%)           |
| 4                                                       | 53 (30.1%)            | 8 (47.1%)           |
| 5                                                       | 10 (5.70%)            | 1 (5.80%)           |
| **MRC sum score**                                       |                       |                     |
| 60–51                                                   | 24 (13.6%)            | 2 (11.8%)           |
| 50–41                                                   | 45 (25.6%)            | 5 (29.4%)           |
| 40–31                                                   | 65 (36.9%)            | 8 (47.0%)           |
| 30–21                                                   | 23 (13.1%)            | 2 (11.8%)           |
| 20–0                                                    | 19 (10.8%)            | 0 (0.00%)           |
| **Sensory deficits**                                    |                       |                     |
| Cranial nerve involvement                               | 13 (7.39%)            | 2 (11.8%)           |
| Acute phase treatment (IVlg/PE)                         | 163 (92.6%)           | 17 (100%)           |

Abbreviations: AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor and sensory axonal neuropathy; GBS, Guillain–Barré Syndrome; IVlg, intravenous immunoglobulins; NCV, nerve conduction velocity; PE, plasma exchange.

3.3 Association between total neutrophils and disease severity

The Pearson correlation was calculated to evaluate the association between total neutrophil counts/ratios and patient MRC sum score, reflecting limb myodynamia. We randomly selected the same number of acute-onset GBS and remission patients as recurrent GBS patients
REN ET AL.

FIGURE 1  (a) The variation of total neutrophil ratios and counts during different GBS courses. (b) Association between total neutrophils and disease severity. Pearson’s correlation was used to analyze the relevance of total neutrophil counts (upper) and total neutrophil ratio (lower) to the Medical Research Council sum score. HCs, healthy controls; GBS, Guillain–Barré syndrome; a-GBS, acute-onset GBS; rem-GBS, GBS in remission; rec-GBS, recurrent GBS (*p < .05, **p < .001)

(n = 17). As shown in Figure 1b, neutrophil counts in the acute-onset GBS group were negatively correlated with their MRC sum scores (r = −0.5657, p = .02). However, the neutrophil ratios in the acute-onset GBS group showed no correlation with the score. There were no significant correlations between neutrophil counts/ratios and MRC sum score in the other groups.

3.4 | Identification of low-density neutrophils and their variation in the whole Guillain–Barré syndrome course

Since LDNs play essential roles in autoimmune disease-derived inflammation, flow cytometry was used for further analysis. LDNs displayed immature and activated characteristics with the expression of CD15+CD11b+CD33+HLA-DR−. Live and antibody-labeled cells were gated and counted (Figure 2a). LDNs accounted for 41.2 ± 1.6%, 55.8 ± 1.9%, 40.3 ± 1.2%, and 54.4 ± 1.9% of peripheral blood cells in the HC, acute-onset GBS, GBS in remission, and recurrent GBS groups, respectively. The cell counts were $1.27 \pm 0.09 \times 10^9$/L, $2.79 \pm 0.24 \times 10^9$/L, $1.33 \pm 0.11 \times 10^9$/L, and $2.89 \pm 0.36 \times 10^9$/L in the above four groups, respectively. The LDN ratios and counts were significantly higher in the acute-onset GBS and recurrent GBS groups compared to the HCs and remission group (Figure 2b).

3.5 | Association between low-density neutrophils and disease severity

We re-evaluated the association between LDN counts/ratios and MRC sum score in the same group of patients. During the acute phase of GBS, LDN counts/ratios were negatively correlated with MRC sum scores (counts: $r = −0.6108, p = .009$; ratio: $r = −0.5372, p = .02$). During remission, the correlation between LDN counts/ratios and MRC sum score was not as significant as that between total neutrophil counts/ratios and MRC sum score (counts: $r = −0.3771, p = .13$; ratio: $r = −0.2551, p = .32$). However, when recurrence occurred, LDN counts/ratios displayed a negative correlation with MRC sum scores...
FIGURE 2  (a) Flow cytometry was used for gating HLA-DR-CD11b+CD15+ CD33+ LDNs. Data were shown as proportions of LDNs in whole blood cells. (b) Variation of LDN ratios and counts during different GBS courses. (c) Association between low-density neutrophils (LDNs) and disease severity. Pearson’s correlation was used to analyze the relevance of LDN counts (upper) and LDN ratios (lower) to the Medical Research Council sum score (*** \( p < .001 \)) (counts: \( r = -0.5081, p = .03 \); ratio: \( r = -0.4917, p = .04 \) (Figure 2c)). This indicates the essential role of LDNs in immune-mediated neuropa-thy compared to the correlation results of total neutrophils.

4 | DISCUSSION

The results of our study showed that LDN ratios and counts in the peripheral blood might serve as potential prognostic indicators in both acute onset and recurrent phases of GBS. Neutrophils play essential roles in the initiation or regulation of local or systemic immune responses, and their hyperactivation is associated with continuous inflammation and tissue damage. Their functions in either GBS or other immune-mediated diseases have been described in previous studies (Hacbarth & Kajdacsy-Balla, 1986; Hassani et al., 2020; Hoffmann et al., 2013; Huang et al., 2018). For instance, neutrophil-to-lymphocyte ratio (NLR) is regarded as a biomarker indicating a GBS pathophysiological or clinical status (Kolaczkowska & Kubes, 2013; Leonhard et al., 2019; Lin et al., 2011). An increased NLR was observed in GBS patients, and a decrease was observed with IVIg treatment. In our study, the neutrophil ratios and counts were elevated in the acute/recurrent phase and maintained at low levels in the remission phase and in healthy
donors. The MRC sum score was used for the comprehensive assessment of patients’ physical status. Neutrophil counts were negatively correlated with MRC sum scores in the acute-onset GBS group, which might indicate that elevation of neutrophils counts leads to worse disease conditions. However, there were no significant correlations between neutrophil counts/ratios and MRC sum scores in the other groups. Although neutrophil counts were significantly higher in recurrent GBS compared to GBS in remission, there was a lack of prognostic value for disease conditions.

Recent studies on neutrophil heterogeneity have identified several subtypes of neutrophils with distinct characteristics. LDNs, initially described as low buoyant density neutrophils (Malide et al., 1995), are considered immature, activated, and degranulated cells with immunomodulatory capabilities (Morisaki et al., 1992; Ng et al., 2019; Ozdemir, 2016). They show increased reactivity to chemotactic factors and inhibitory effects on T cell proliferation and natural killer cell activation (Platek et al., 2018; Rahman et al., 2019). They are also characterized by decreased phagocytic capacity and impaired reactive oxygen species production (Rosales, 2020). Inflammatory processes, such as degranulation, might contribute to buoyant density loss. It has been reported that LDNs secrete higher levels of pro-inflammatory cytokines, including interleukin 8 (IL-8), IL-6, type I interferons, and tumor necrosis factor-α (Ruts et al., 2010; Saeed et al., 2019). Flow cytometry was used for analysis based on the cell surface markers. LDNs expressed low CD14 but high CD15, CD11b, and CD33, with a lack of major histocompatibility complex class II (Ruts et al., 2010; Sagiv et al., 2015). To date, LDNs have been reported in many immune-mediated diseases such as SLE, psoriasis, vasculitis, sepsis, human immunodeficiency virus infection, and pristane-induced arthritis (Grayson et al., 2015; Hacbarth & Kajdacsy-Balla, 1986; Shahrizaila et al., 2021; Spiegel et al., 2016; Tay et al., 2020). LDNs are regarded as potentially pathogenic cell types or prognostic indicators throughout the above-mentioned disease courses. However, in GBS, which is previously described as an adaptive immune disease, neutrophils detections are not valued as B lymphocytes or antibodies. In our study, we detected LDNs in different clinical courses of GBS patients for the first time and observed the variation tendency of LDNs in acute-onset and recurrent phases of GBS in particular. After cell gating with a certain flow cytometry strategy, both LDN ratios and counts showed significant differences along with changes in disease conditions. They increased in acute-onset GBS and decreased to basal levels in the remission phase. In recurrent GBS, the LDN counts and ratios were again elevated significantly. Further assessment of the associations between LDNs and MRC sum scores of patients in different phases indicated that LDNs were specifically correlated with disease recurrence. As the number of LDNs increased, patients experienced clinical deterioration in symptoms and signs. Immediate IVIg retreatment was administered to these patients, and improvements were observed (not shown).

The indications for GBS recurrence have remained controversial worldwide. For instance, autoimmune anti-ganglioside antibodies are frequently detected for disease assessment and prognosis, since GBS is generally recognized as a humoral immune-mediated disease. However, the antibodies might not be as vital as expected. They were neither used as alternative diagnostic criteria nor regarded as indicators of clinical fluctuations (Uncini & Kuwabara, 2012; van Koningsveld et al., 2007; Wang et al., 2018). The initiating factors or other underlying mechanisms of GBS recurrence remain unclear, complicating prognostic estimation. Our findings showed that, as an accessible immunological indicator, LDN variation might be correlated with GBS progression and disease severity in different phases. However, larger sample sizes and extended follow-up time are needed for further clinical studies, since the number of recurrent patients is small in the present study. Additional studies focused on treatment influence and LDNs modulatory mechanisms are yet to be conducted.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
All data included in this study are available upon request by contact with the corresponding author.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/brb3.2456

ORCID
Jun Guo https://orcid.org/0000-0001-8053-881X

REFERENCES
Asbury, A. K., & Cornblath, D. R. (1990). Assessment of current diagnostic criteria for Guillain-Barré syndrome. Annals of Neurology, 27(Suppl), S21–24.
Carmona-Rivera, C., & Kaplan, M. J. (2013). Low-density granulocytes: A distinct class of neutrophils in systemic autoimmunity. Seminars in Immunopathology, 35(4), 455–463.
Cassery, C. S., Nantes, J. C., Whitaker Hawkins, R. F., & Vallières, L. (2017). Neutrophil perversion in demyelinating autoimmune diseases: Mechanisms to medicine. Autoimmunity Reviews, 16(3), 294–307.
Cloke, T., Munder, M., Taylor, G., Müller, I., & Kropf, P. (2012). Characterization of a novel population of low-density granulocytes associated with disease severity in HIV-1 infection. Plos One, 7(11), e48939.
Coffelt, S. B., Kersten, K., Doornebal, C. W., Weiden, J., Vrijland, K., Hau, C. S., Verstegen, N. J. M., Ciampicotti, M., Hawinkels, L. J. A. C., Jonkers, J., & deVisser, K. E. (2015). IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature, 522(7556), 345–348.
Denny, M. F., Yalavarthi, S., Zhao, W., Thacker, S. G., Anderson, M., Sandy, A. R., McCune, W. J., & Kaplan, M. J. (2010). A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. Journal of Immunology, 184(6), 3284–3297.
Dritze, G., Dunn-Siegrist, I., Tissières, P., & Pugin, J. (2013). Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. Critical Care Medicine, 41(3), 820–832.
Fortunati, E., Kazemier, K. M., Grutters, J. C., Koenderman, L., & Van den Bosch, V. J. (2009). Human neutrophils switch to an activated phenotype after homing to the lung irrespective of inflammatory disease. Clinical and Experimental Immunology, 155(3), 559–566.
Fujimi, A., Ikeda, Y., Ono, K., & Kanisawa, Y. (2012). Botryoid nuclei of neutrophils and monocytes in autoimmune limbic encephalitis. International Journal of Hematology, 95(4), 329–320.

Goodfellow, J. A., & Willson, H. J. (2016). Guillain-Barré syndrome: A century of progress. Nature Reviews Neurology, 12(12), 723–731.

Grand'Maison, F., Feasby, T. E., Hahn, A. F., & Koopman, W. J. (1992). Recurrent Guillain-Barré syndrome. Clinical and laboratory features. Brain, 115(Pt 4), 1093–1106.

Grayson, P. C., Carmona-Rivera, C., Xu, L., Lim, N., Gao, Z., Asare, A. L., Specks, U., Stone, J. H., Seo, P., Spiera, R. F., Langford, C. A., Hoffman, G. S., Kallenberg, C. G., St Clair, E. W., Tchao, N. K., Ytterberg, S. R., Phippard, D. J., Mekel, P. A., Kaplan, M. J., & Monach, P. A. (2015). Rituximab in ANCA-associated vasculitis-immune tolerance network research group. Neutrophil-related gene expression and low-density granulocytes associated with disease activity and response to treatment in antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis and Rheumatology, 67(7), 1922–1932.

Habarth, E., & Kajdacsy-Balla, A. (1986). Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. Arthritis and Rheumatism, 29(11), 1334–1342.

Hassani, M., Hellebrekers, P., Chen, N., vanAalst, C., Bongers, S., Hietbrink, F., Koenderman, L., & Vrisekoop, N. (2020). On the origin of low-density neutrophils. Journal of Leukocyte Biology, 107(5), 809–818.

Hoffmann, M. H., Bruns, H., Bäckdahl, L., Neregård, P., Niederreiter, B., Her mann, M., Catrina, A. I., Agerberth, B., & Holmdahl, R. (2013). The cathe licidins LL-37 and rCRAMP are associated with pathogenic events of arthritis in humans and rats. Annals of the Rheumatic Diseases, 72(7), 1239–1248.

Huang, Y., Ying, Z., Quan, W., Xiang, W., Xie, D., Weng, Y., Li, X., Li, J., & Zhang, X. (2018). The clinical significance of neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio in Guillain-Barré syndrome. International Journal of Neuroscience, 128(8), 729–735.

Hüner, E. A., Dai, A. I., & Demiryürek, A. T. (2018). Association of neutrophil-to-lymphocyte ratio with intravenous immunoglobulin treatment in children with Guillain-Barré syndrome. Journal of Child Neurology, 33(2), 164–167.

Inoue, N., Kunishige, M., Yoshida, S., Oshima, Y., Ohnishi, Y., Kuroda, Y., Asano, A., Yoshino, H., Matsumoto, T., & Mitsui, T. (2003). Dissociation between titer of anti-ganglioside antibody and severity of symptoms in a case of Guillain-Barré syndrome with treatment-related fluctuation. Journal of the Neurological Sciences, 210(1–2), 105–108.

Kieseler, B. C., Matthey, E. K., Sommer, C., & Hartung, H. P. (2018). Immune-mediated neuropathies. Nature Reviews Disease primers, 4(1), 31.

Kolaczkowska, E., & Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. Nature Reviews Immunology, 13(3), 159–175.

Leonhard, S. E., Mandaracas, M. R., Gondim, F. A. F., Bateman, K., Ferreira, M. L. B., Cornblath, D. R., vanDoorn, P. A., Dourado, M. E., Hughes, R. A. C., Islam, B., Kusunoki, S., Pardo, C. A., Reisin, R., Sejvar, J. J., Shahrizaila, N., Soares, C., Umapinha, T., Wang, Y., Yiu, E., ... Jacobs, B. C. (2019). Diagnosis and management of Guillain-Barré syndrome in ten steps. Nature Reviews Neurology, 15(11), 671–683.

Lin, A. M., Rubin, C. J., Khandpur, R., Wang, J. Y., Riblett, M., Yalavathri, S., Vil lanueva, E. C., Shah, P., Kaplan, M. J., & Bruce, A. T. (2011). Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. Journal of Immunology, 187(1), 490–500.

Malide, D., Russo, P., & Bendayan, M. (1995). Presence of tumor necrosis factor alpha and interleukin-6 in renal mesangial cells of lupus nephritis patients. Human Pathology, 26(5), 558–564.

Morisaki, T., Goya, T., Ishimitsu, T., & Torisu, M. (1992). The increase of low density subpopulations and CD10 (CALLA) negative neutrophils in severely infected patients. Surgery Today, 22(4), 322–327.

Ng, L. G., Ostuni, R., & Hidalgo, A. (2019). Heterogeneity of neutrophils. Nature Reviews Immunology, 19(4), 255–265.

Ozdemir, H. H. (2016). Analysis of the albumin level, neutrophil-lymphocyte ratio, and platelet-lymphocyte ratio in Guillain-Barré syndrome. Arquivos De Neuro-Psiquiatria, 74(9), 718–722.

Piatek, P., Domowicz, M., Przygodzka, P., Matysiak, M., Dziiko, K., & Lewkowicz, P. (2018). CSa-pretreated neutrophils are critical for autoimmune-induced astrocyte dysregulation in neuromyelitis optica spectrum disorder. Frontiers in Immunology, 9, 1694.

Ramzan, S., Sagar, D., Hanna, R. N., Lightfoot, Y. L., Mistry, P., Smith, C. K., Manna, Z., Hasni, S., Siegel, R. M., Sanjuan, M. A., Kolbeck, R., Kaplan, M. J., & Casey, K. A. (2019). Low-density granulocytes activate T cells and demonstrate a non-suppressive role in systemic lupus erythematosus. Annals of the Rheumatic Diseases, 78(7), 957–966.

Rosales, C. (2020). Neutrophils at the crossroads of innate and adaptive immunity. Journal of Leukocyte Biology, 108(1), 377–396.

Ruts, L., Drenth, J., Jacobs, B. C., & vanDoorn, P. A., Dutch GBS Study Group. (2010). Distinguishing acute-onset CIDP from fluctuating Guillain-Barre syndrome: A prospective study. Neurology, 74(21), 1680–1686.

Saeed, M. L., Kaleem Baloch, B., Mahmoud, S. N., Khan, M. T., Qureshi, M. S. S., Shad, Z. S., Hussain, S. W., Munawar, K., Qadeer, A., & Abdul lah, A. (2019). Role of anti-ganglioside antibodies in the diagnosis of Guillain-Barré syndrome as an alternate investigation. Cureus, 11(5), e4625.

Sagiv, J. Y., Michaeli, J., Assi, S., Mishalian, I., Kisos, H., Levy, L., Damti, P., Lum broso, D., Polyansky, L., Sionov, R. V., Ariel, A., Hovav, A. H., Henke, E., Fridlinger, Z. G., & Granot, Z. (2015). Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Reports, 10(4), 562–573.

Shahrizaila, N., Lehmann, H. C., & Kuwabara, S. (2021). Guillain-Barré syn drome. Lancet, 397(10028), 1214–1228.

Spiegel, A., Brooks, M. W., Houshyar, S., Reinhardt, F., Ardolino, M., Fessler, E., Chen, M. B., Krall, J. A., DeCock, J., Zervantonakis, I. K., Iannello, A., Iwamoto, Y., Cortez-Retamozo, V., Kamm, R. D., Pitett, M. J., Rautel, D. H., & Weinberg, R. A. (2016). Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. Cancer Discovery, 6(6), 630–649.

Tay, S. H., Celhar, T., & Fairhurst, A. M. (2020). Low-density neutrophils in systemic lupus erythematosus. Arthritis & Rheumatology, 72(10), 1587–1595.

Uncini, A., & Kuwabara, S. (2012). Electrodiagnostic criteria for Guillain-Barré syndrome: A critical revision and the need for an update. Clinical Neurophysiology, 123(8), 1487–1495.

vanKoningsveld, R., Steyerberg, E. W., Hughes, R. A., Swan, A. V., van Doorn, P. A., & Jacobs, B. C. (2007). A clinical prognostic scoring system for Guillain-Barré syndrome. Lancet Neurology, 6(7), 589–594.

Wang, X., Qiu, L., Li, Z., Wang, X. Y., & Yi, H. (2018). Understanding the multi faceted role of neutrophils in cancer and autoimmune diseases. Frontiers in Immunology, 9, 2456.

---

How to cite this article: Ren, K., Yang, A., Lu, J., Zhao, D., Bai, M., Ding, J., Wei, T., Li, H., & Guo, J. (2022). Association between serum low-density neutrophils and acute-onset and recurrent Guillain-Barré syndrome. Brain and Behavior, 12, e2456. https://doi.org/10.1002/brb3.2456