



Sexual dimorphism in neutrophil function: Unveiling the discriminative nature of male and female neutrophils

Richard F. Kraus^{a,*}, Nina Doblinger^b, Michael A. Gruber^a, Maria S. Wagner^c, Johanna Rosenberger^a

^a Department of Anesthesiology, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

^b Department of Internal Medicine I, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

^c Department of Cardiothoracic Surgery, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

ARTICLE INFO

Editor: Dr. Y Ali

Keywords:

Neutrophil function

Chemotaxis

Sexual dimorphism

Epitopes

Sex

ABSTRACT

Background: Women and men are different on many biological levels. Mounting evidence is now recognized that even the immune system has some significant sex differences, which are mainly cell mediated. This study investigated sex-specific differences in function and regulation of polymorphonuclear neutrophil granulocytes (PMNs) in male and female healthy human donors to gain a deeper understanding of the immune response and potential sex-specific dimorphism in immunology.

Methods: PMNs were obtained from whole blood samples of healthy female and male donors by leuco-/lymphospin density centrifugation. Chemotaxis assays using μ -slide chemotaxis chambers were performed, in which N-formylmethionin-leucyl-phenylalanine (fMLP) stimulated PMNs migrated through a type I collagen matrix. We measured the production of reactive oxygen species (ROS), the release of myeloperoxidase (MPO), and the formation of Neutrophil Extracellular Traps (NETs). Additionally, a flow cytometry assay was conducted to examine functional variations of neutrophil surface markers CD62L, CD11b, and CD66b, as well as the oxidative burst in PMNs obtained from male and female donors.

Results: Sex specific differences of neutrophil function could be determined. Male-derived PMNs initially migrated further distances, while female-derived PMNs showed more targeted movement. However, as the observation period progressed, male-derived PMNs began to exhibit more targeted migration, maintaining straightness towards the end. Differences in neutrophil surface marker expression were observed, with greater levels of CD11b and CD66b on male-derived PMNs after 2 h resting. The different immune effects between the sexes were seen in live cell imaging as well as in flow cytometry analyses.

Conclusion: The study revealed significant functional differences between PMNs from male and female donors. To gain reliable results in future PMN studies, it is crucial to consider the sex of the donor.

1. Introduction

The human immune system displays notable variations between males and females. There is growing evidence that, even outside of reproductive organs, biological sex influences the innate immune system through genetic disparities and also variations in sex hormones. This leads to sexual dimorphism in immune processes (see Fig. 1, (Jaillon et al., 2019; Libert et al., 2010)).

The major genetic changes involved in differences existing between male and female innate immune response reside in the X chromosome (Jaillon et al., 2019; Libert et al., 2010). In fact, the X chromosome

carries different genes that are involved in innate disorders, such as genes escaping X chromosome inactivation and cellular mosaicism (Jaillon et al., 2019; Chuang et al., 2009). X-linked primary immunodeficiencies are genetic disorders of the immune system that predominantly affect males. In the context of the innate immune response, a mutation in the NOX2 gene, essential for triggering the intracellular oxidative burst necessary for phagocytes, such as polymorphonuclear neutrophil granulocytes (PMNs), to eliminate microorganisms, leads to X-linked chronic granulomatous disease (CGD). Individuals with this condition are particularly vulnerable to frequent bacterial, fungal and viral infections (Jaillon et al., 2019).

* Corresponding author.

E-mail address: Richard-Felix.Kraus@klinik.uni-regensburg.de (R.F. Kraus).

<https://doi.org/10.1016/j.imbio.2025.153128>

Received 28 April 2025; Received in revised form 16 September 2025; Accepted 15 October 2025

Available online 20 October 2025

0171-2985/© 2025 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

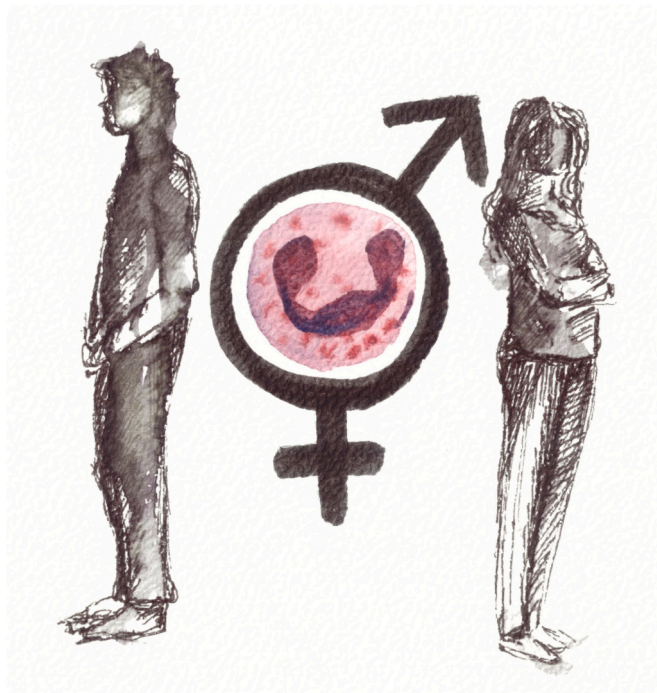


Fig. 1. In the human (innate) immune system, a sexual dimorphism is increasingly emerging, which appears to be primarily cell-mediated. Neutrophils are the most important component of the innate immune system and seem to play a central role.

However, not only in congenital defects, but also in healthy individuals, there is growing evidence that sexual dimorphism of the innate immune response exists, which is mediated most notably at the cellular level. In the case of PMNs, the most important cellular component of the innate immune system, a cellular difference between female- and male-derived cells can even be recognized histologically (see Fig. 2).

Evidence for a cellular difference between PMNs from phenotypically female and male mammals (in the following described as “female” and “male” PMNs) is based on an observation from the early 2000s. This revealed that female rat PMNs were more resistant to activation by burns or plasma from trauma or hemorrhagic subjects than the male equivalents (Deitch et al., 2006). Since then, research into sexual immune dimorphism has revealed many differences between male and female PMNs. In female PMNs, for instance, a reduced spontaneous apoptosis compared to male PMNs has been observed (Shepherd et al., 2020; Molloy et al., 2003). Furthermore, young (20 to 30 years old) healthy adult males, displayed a significant enhancement in an immature neutrophil gene signature compared to age-matched females (Gupta et al., 2020). The ITGAX and FCGR3A genes (encoding for CD11b and CD66b as markers for neutrophil activation surface epitopes) were found to be downregulated in immature PMNs compared with mature ones (Blazkova et al., 2017). There is a difference in the transcriptional profile of female PMNs compared to male PMNs. 106 genes were observed to be up-regulated and 128 genes to be downregulated in female PMNs compared to male neutrophils. It is therefore important to note that PMNs in earlier stages of development are more transcriptionally active. Primary granule genes, which are expressed at higher levels during the early stages of PMN development, were higher in male than in female PMNs, suggesting a more immature phenotype (Gupta et al., 2020).

Moreover, healthy young adult females exhibit an activated/mature neutrophil profile characterized by enhanced type I interferon pathway activity, enhanced proinflammatory responses, and distinct bioenergetics (Gupta et al., 2020).

Peripheral blood PMNs appeared to be more mature and responsive

in young females, who also exhibited an elevated capacity formation of Neutrophil Extracellular Traps (NETs) at baseline and upon microbial or sterile autoimmune stimuli (Blazkova et al., 2017). Conversely, PMNs during pregnancy showed increased expression of CD15 and decreased expression of CD10 - which is characteristic of less mature neutrophils (Blazkova et al., 2017).

Furthermore, blood transfusion products from female donors are at risk of developing antibodies against neutrophil surface structures (known as anti-human-neutrophil-antigen antibodies (anti-HNA)), which are partly responsible for one of the most serious adverse effect in recipients of blood transfusions, the transfusion-related acute lung injury (TRALI) (Schönbacher et al., 2021; Peters et al., 2015).

Anti-HNA antibody production can occur when women are exposed to foreign antigens of their foetus during pregnancy. If these “primed” women then donate blood after pregnancy, their donated plasma may contain anti-HNA antibodies (Peters et al., 2015). If a patient subsequently receives a transfusion of blood plasma containing such antibodies against PMN surfaces, this can trigger TRALI. The key initiation steps of TRALI involve the priming and activation of neutrophils due to anti-HNA. Therefore, also from this point of view special attention should be paid to sex-specific differences, with a focus on exploring the unique nuances that exist between male and female donors (Liu et al., 2023).

In summary, accumulated studies suggest that aspects of neutrophil biology exhibit sex dimorphism. However, the pathways underlying sexual dimorphism in neutrophils, and the extent to which it occurs, remain unclear (Lu et al., 2021). Importantly, the mechanisms driving these contrasts between male and female neutrophil response remain insufficiently characterized (Gupta et al., 2020). To further characterize the functional and molecular differences between male and female PMNs, we performed functional testing by live cell imaging and a molecular analysis of surface epitopes by flow cytometry of male and female PMNs. We examined CD11b, CD66b and CD62L due to their important roles in indicating PMN activation status and enabling PMNs to fulfill their functions. Upregulation of CD11b facilitates the adhesion of activated PMNs to the vascular endothelium and promotes their subsequent transmigration across the blood vessel wall (Kraus and Gruber, 2021a). CD66b, a member of the carcinoembryonic antigen family, functions as a granulocyte-specific receptor primarily involved in mediating phagocytosis (Lakschevitz et al., 2016). CD62L is crucial for extravasation across blood vessels and is consequently expressed at higher levels in the bloodstream. Moreover, by this surface epitopes we examined the PMN activation status (Kraus and Gruber, 2021a).

2. Materials and methods

2.1. Vote of the ethics committee

For the implementation of this study, results of several projects were summarized. The conduct of the projects was in line with the principles of the Declaration of Helsinki and were approved by the local Ethics Committee of the Medical Faculty at the University of Regensburg (vote numbers 16-101-0322, 18-1210-10 and 18-1025-101).

2.2. Sample collection and isolation of neutrophil granulocytes

After obtaining informed consent 7.5 mL of whole blood was taken from a suitable antecubital vein into a lithium heparin blood collection tube using a safety multifly cannula from healthy Caucasian volunteers (demographic data is summarized in Table S1 & S2 in the supplement). Thereby, an age-matched selection of female and male test persons was accomplished, with sex determined based on external phenotype. Chromosome analyses were not performed. For live cell imaging (see section 2.3) a total of $n = 12$ (5 female and 7 male) samples were examined. For flow cytometry the total was $n = 97$ (48 female and 49 male samples). Blood samples were all taken in the morning (08.00 to

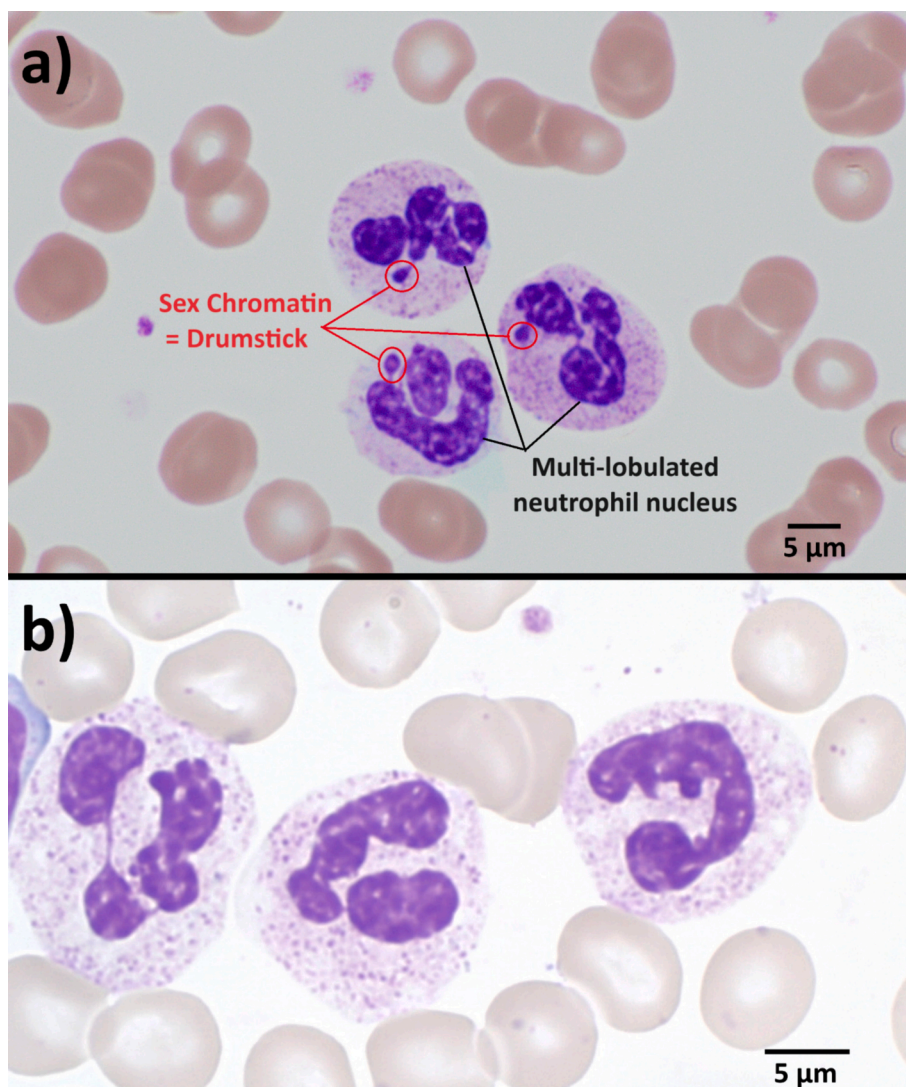


Fig. 2. a) Blood smear in Pappenheim stain of a female person. The drumstick is clearly recognizable: A neutrophil's drumstick is a small projection of chromatin that is attached to the nucleus. It is also known as "Barr body" and is typically found in the nuclei of female neutrophils. The presence of a drumstick is result of X-chromosome inactivation (Graphic provided by the Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany). b) Male segmented neutrophil granulocytes in Pappenheim-stained blood cell smears (graphic provided by the laboratory for Paediatric Oncology and Haematology at the University Hospital Regensburg, Germany). Both images were originally taken at 1000 $\times$ magnification by light microscopy, digitally enlarged and edited.

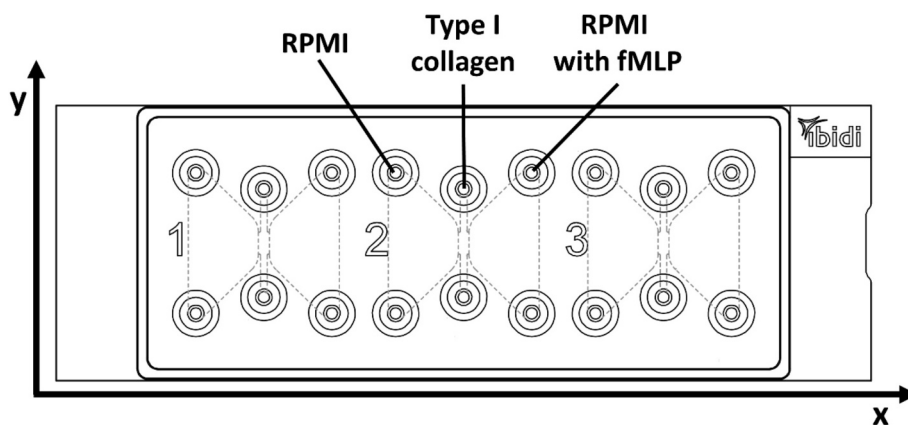


Fig. 3. Experimental setup μ -slide chemotaxis chamber with three examination positions (here marked by numbers). At each position there was a canal with collagen matrix in the middle and two adjacent reservoirs filled with RPMI resp. RPMI with fMLP (Graphic provided by IBIDI GmbH Martinsried).

10.00 am) (Kraus et al., 2021).

PMNs were separated at room temperature using density gradient centrifugation for 20 min at 756 g by layering PBMC (Lympho) Spin Medium on top of Leuko Spin Medium (both from pluriSelect Life Science). They were then washed once and resuspended in fetal calf serum to obtain a solution at a concentration of 18×10^6 cells/mL as previously described (Kraus et al., 2021; Doblinger et al., 2019).

2.3. Live cell imaging

2.3.1. Preparation of the μ -Slide chemotaxis chambers

For live cell imaging a 3D- μ -Slide chemotaxis device (IBIDI GmbH, Graefelfing, Germany) was used. At each of the three positions, these chambers have a canal in the middle, which was filled with a type I collagen matrix mixed with the isolated PMNs as described in several publications by our research group (see Fig. 3) (Kraus et al., 2021; Doblinger et al., 2019; Pai et al., 2020). On either side of the canal were two reservoirs. The reservoir on the left consistently contained a 10 nM solution of N-formylmethionin-leucyl-phenylalanine (fMLP) throughout all experiments. Similarly, the reservoir on the right was filled with RPMI medium once the canal matrix had set. This setup established a gradient for chemotaxis (Kraus et al., 2021).

2.3.2. Microscopy and live cell imaging

The IBIDI Heating and Gas Incubation System (IBIDI GmbH, Martinsried, Germany) was used to establish an experimental setting maintained at 37 °C, with 5 % CO2 and 50 % humidity for microscopic analysis. Reactive oxygen species (ROS) generation was detected using 1 μ M dihydrorhodamine 123 (DHR 123, Life Technologies - Thermo Fisher Scientific). To visualize NET formation, a DNA stain, 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich), was applied at a concentration of 0.5 μ g/mL. The release of myeloperoxidase (MPO) was assessed using 0.5 μ g/mL of an APC-conjugated anti-human MPO antibody (REA491, Miltenyi Biotec) (Kraus et al., 2021). Migration and fluorescence were evaluated following a protocol outlined by Doblinger et al. using a DMI8 inverted microscope (Leica Microsystems, Wetzlar, Germany) (Doblinger et al., 2019). Over a period of up to six hours, fluorescence and phase contrast images were captured automatically at a magnification of 100 $\times$ using a DFC9000 GT SC CMOS black and white camera (Leica Microsystems). This was accomplished with the Application Suite X software platform (Version 3.4.2.18368, Leica Microsystems), taking a photograph every 30 s, resulting in the collection of 720 individual images documenting the activities within each IBIDI channel (Kraus et al., 2021).

2.3.3. Analysis of image data

The series of images produced by the microscope were examined using Imaris® software (Version 9.0.2, Bitplane AG). To assess migration, the x and y axes were defined for each channel throughout the entire relevant image area, similar to a Cartesian coordinate system (see Fig. 3). This enabled the determination of precise migration metrics (see Table 1 and Fig. 4).

The 30-min observation periods were adjusted based on the timing of

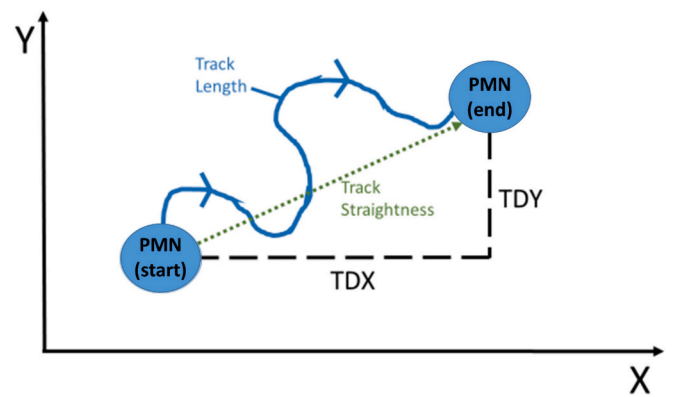


Fig. 4. Illustration of the migration parameters: TL = Track Length, TDX = Track Displacement Length X, TDY = Track Displacement Length Y within the set Cartesian coordinate system.

gel-cell interaction. In this process, filters were applied to ensure that Track Duration was >1000 s and Tracklength >10 μ m (Kraus et al., 2021).

Two distinct evaluation approaches were employed to analyze migration. In the initial analytical approach, all cells were regarded as distinct units, with each single PMN cell entered into the analysis as an individual entity. In the second approach, the cells were considered “technical replicates”, with all cells from a given test person combined and examined as a group.

All dyes utilized to measure NETs, MPO and ROS were visualized as colored regions in the fluorescence images. These were automatically identified by Imaris® in each single image and exported to a separate Excel file containing the areas [μ m²] of each specific color surface in every image. The cumulative areas at each time point were aggregated in Excel. The assessment of ROS generation using DHR 123 exhibited a parabolic trend. The time taken to reach maximum ROS production, (T_{max}ROS), was determined using a third-degree polynomial trendline on the relevant section of the graph. The first derivative of this equation and its zero point were computed (see Fig. 5) (Doblinger et al., 2019).

NET quantification with DAPI and anti-MPO resulted in sigmoidal curves (see Fig. 6). The time to achieve half-maximum effect, ET₅₀, was calculated using Phoenix NLME (Certara Inc., see Fig. 6) (Doblinger et al., 2019).

2.3.4. Flow cytometry

The levels of neutrophil cell-surface antigen expression and the respiratory burst were analyzed using flow cytometry, employing a FACSCalibur flow cytometer along with CellQuest Pro Software (Version 5.2), both provided by BD Biosciences, as outlined previously (Doblinger et al., 2019; Kraus et al., 2024a). The respiratory burst was assessed using 20 μ L of preincubated cells suspended in 1 mL of Dulbecco’s phosphate-buffered saline (DPBS, Sigma-Aldrich), along with 10 μ L of DHR 123 (10 μ M) and 10 μ L of the fluorescent dye seminaphtharhodafluor (SNARF) at a concentration of 10 μ M (Invitrogen).

Table 1
Parameters recorded for quantifying migration via live-cell imaging.

Track Displacement X (TDX) [μ m]	covered distance in direction x
Track Displacement Y (TDY) [μ m]	covered distance in direction y
Track Length (TL) [μ m]	effectively covered distance
Track Displacement Length (TDL) [μ m]	Euclidean distance between the start and the end point
Track Straightness (TS) = $\frac{\text{Track Displacement Length}}{\text{Track Length}}$	
Track Duration [s]	duration of ‘tracked’ chemotactic migration

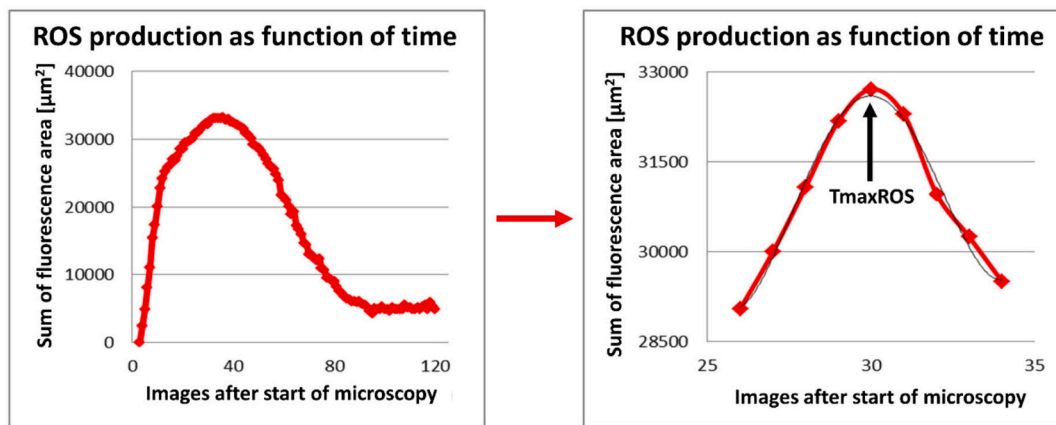


Fig. 5. Schematic illustration of the relationship of ROS production over the total experimental time (left) and selection of the maximum range (right) with representation of the calculation of a third-degree polynomial (trend line) to determine $T_{\max\text{ROS}}$ by mathematic derivation and determination of the zero point.

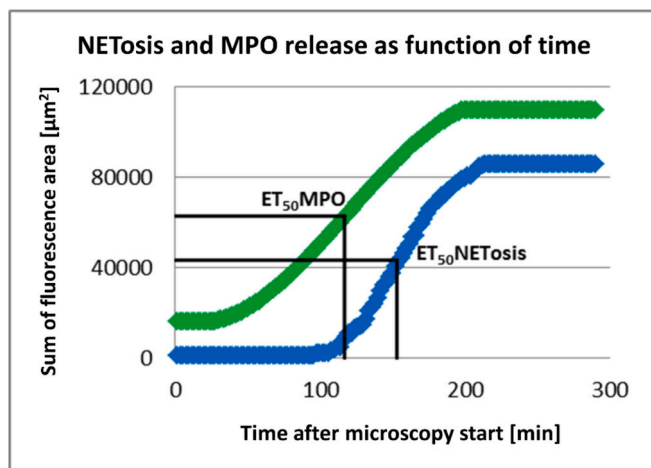


Fig. 6. Graphical (schematic) description for calculating the ET_{50} -values of NETs and MPO release by Phoenix®.

The respiratory burst was triggered by adding either 10 μL of fMLP (10 μM) combined with 10 μL of human tumor necrosis factor alpha ($\text{TNF}\alpha$, 1 $\mu\text{g}/\text{mL}$, PeproTech Inc.) or 10 μL of phorbol-12-myristate-13-acetate (PMA, 10 μM , Sigma-Aldrich). For immunophenotyping purposes, the expression of various antigens on the surface of PMNs was analyzed using antibodies tagged with phycoerythrin (PE), fluorescein isothiocyanate (FITC), or allophycocyanin (APC) for fluorescence detection (Doblinger et al., 2019; Kraus et al., 2024a). The following antibodies were utilized: anti-CD11b conjugated with PE (ICRF44, BioLegend), anti-CD62L conjugated with FITC (DREG-65, BioLegend), anti-CD66b conjugated with APC (G10F5, BioLegend), and anti-LOX-1 also conjugated with APC (130-122-111, MiltenyiBiotec, Bergisch Gladbach, Germany). The data obtained from these measurements were evaluated using FlowJo (Version 10.0.7; FlowJo LLC) (Doblinger et al., 2019; Pai et al., 2020; Kraus et al., 2024a). The gating strategies are shown in Fig. S1 in the supplement.

2.3.5. Statistical analysis

Statistical evaluations were performed using SPSS® Statistics 29 (IBM). The normality of the data distribution was assessed through the Kolmogorov-Smirnov test. Where the data exhibited a normal distribution, and homogeneity of variance was present during multiple comparisons, mean values (MV) were analyzed for significant differences using a one-way analysis of variance (ANOVA), with standard deviations reported as $\pm$ SD. In cases where homogeneity of variance

was not present, Welch's ANOVA was employed. For datasets demonstrating homogeneity of variance, post hoc analyses were conducted using the Bonferroni method. Conversely, when variance was found to be heterogeneous, the Dunnett's T3 test was utilized. Levene's test was applied to evaluate homogeneity of variance. When the data did not conform to a normal distribution, central tendencies of the groups were compared using the Kruskal-Wallis test and the results were reported as medians and interquartile ranges. The subsequent post hoc analysis for this test was carried out using Dunn-Bonferroni testing. A significance level of $p < 0.05$ was considered statistically significant. The distribution of results is illustrated through simple or grouped boxplots, displaying the median, interquartile range, and calculated minimum and maximum values. Statistical outliers are indicated by circles and extreme values by asterisks (Kraus et al., 2021; Kraus et al., 2024a).

3. Results

3.1. Live cell imaging – migration analysis

More detailed results of the statistical analysis of migration can be found in Table S5 in the supplement.

3.1.1. Individual cell-based evaluation

3.1.1.1. Male PMNs cover longer distances. As indicated by greater values for parameter TrackLength, male PMNs migrated further distances in every time slot than female PMNs (see Fig. 7 and Table 2).

3.1.1.2. Male PMNs move further away from the origin. In every time slot male PMNs moved further away from their starting point as indicated by greater values for parameter TrackDisplacementLength in male than in female PMNs (see Fig. 8 and Table 3).

3.1.1.3. Female PMNs migration more targeted at the beginning. At the start of the experiments, female PMNs migrated in a more targeted way than male PMNs as indicated by greater values for parameter TrackStraightness ($p = 0.04$, see Fig. 9). In the second time slot (31–60), both migrated equally targeted ($p = 0.955$). However in the third time slot (61–90) male PMNs began to migrate more targeted, maintaining straightness towards the end despite minimal overall migration (see Fig. 9 and Table 4).

3.1.1.4. Intact chemotaxis gradient in all experiments. The values obtained for TrackDisplacementX and TrackDisplacementY indicated the primary movement of neutrophils with a focus on the positive X direction, aligning with the direction of the fMLP gradient. The medians of

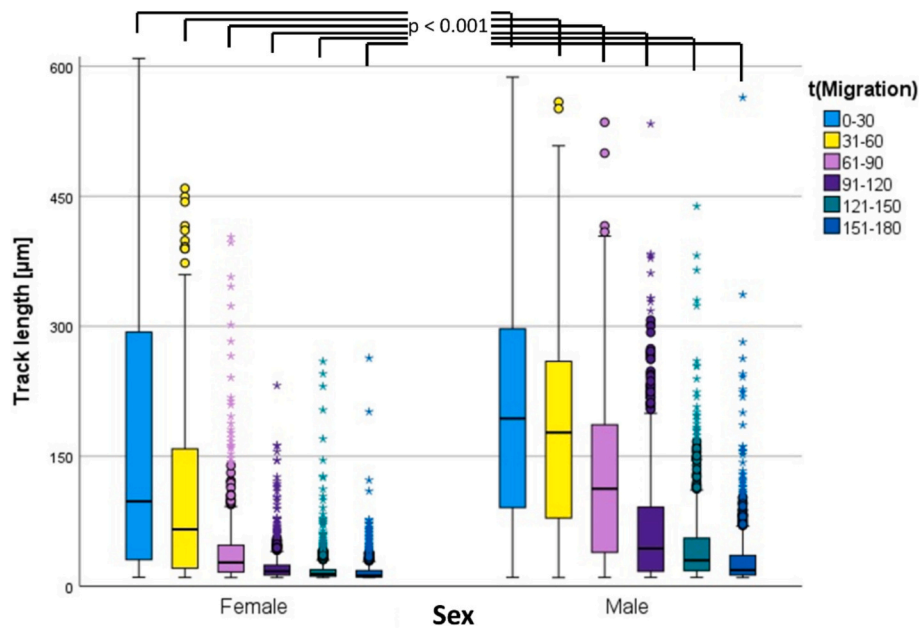


Fig. 7. Overview of migration distances of PMN (TrackLength [μm]), split in observation periods of 30 min, and sex. Overall migration decreased towards the end of the observation period. The grouped box plot presents the results of $n = 12$ experiments, using medians with interquartile range. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 2
Overview of medians and interquartile ranges (round brackets) of migration lengths (TrackLength TL) of the different sexes (individual cell number (n) in square brackets) in determined 30 min time sections, which could be observed by means of live-cell imaging.

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female TL	97.8 μm (263.4 μm) [$n = 725$]	65.6 μm (137.9 μm) [$n = 687$]	27.4 μm (40.0 μm) [$n = 743$]	17.4 μm (11.3 μm) [$n = 734$]	14.0 μm (7.7 μm) [$n = 752$]	12.3 μm (7.4 μm) [$n = 663$]
	193.5 μm (206.6 μm) [$n = 930$]	177.2 μm (181.4 μm) [$n = 1067$]	112.5 μm (147.3 μm) [$n = 1044$]	43.5 μm (74.2 μm) [$n = 1029$]	29.9 μm (37.5 μm) [$n = 797$]	18.6 μm (22.7 μm) [$n = 778$]
Male TL						

TDX had a positive value in every time slot -with exception of time slot (61–90 with TDX = $-0.1 \mu\text{m}$ [IQR $3.1 \mu\text{m}$]; see Table S3 and Fig. S2 in the supplement). The medians of the parameter TDY were around zero, ranging from 0.0 to $+1.7 \mu\text{m}$ (see Table S4 and Fig. S3 in the supplement).

3.1.2. Aggregation of cells as technical replicates for combined evaluation

3.1.2.1. Male PMNs cover longer distances in the middle time slots. In every time slot male PMNs migrated further distances (reaching significance in time slot 31–60 and 121–150; see Fig. 10 and Table 5).

3.1.2.2. Male PMNs showed tendency to move further away from the origin. Initially, the female PMNs exhibited a tendency to move more distant from the origin as indicated by the greater mean TrackDisplacementLength. However, as the experiments progressed, a notable shift was observed. From time slot 31–60 the male PMNs moved further away from the origin, reaching statistical significance by the end of the experiment (see Fig. 11 and Table 6).

3.1.2.3. Female PMNs migration showed tendency for more targeted at the beginning. In the beginning of the experiments female PMNs showed tendency for a more targeted way than male PMNs. This turned in the end of the experiments where male PMNs exhibited more targeted migration, though this difference was not statistically significant (see Fig. 12 and Table 7).

3.2. Live cell imaging: no difference in immune functions

No differences were found in the immune effects between male and female PMNs (see Table 8): ($p = 0.68$ for MeanValues(T_{maxROS}); $p = 0.24$ for MeanValus($ET_{50}\text{MPO}$); $p = 1.0$ for MeanValues($ET_{50}\text{NETosis}$).

3.3. Results of flow cytometry

Flow cytometry analysis acquired 10,000 cells per sample, of which 16–99 % were identified as PMNs. The mean PMN proportion across the antibody staining samples was 66 % ($\pm 24.9 \%$), which remained consistent with the rhodamine 123 testing assays, which averaged 66 % ($\pm 21.4 \%$). More detailed results of the statistical analysis of the flow cytometry can be found in Table S6 of the supplement.

3.3.1. Results of CD11b

Immediately after isolation (= without resting), the mean fluorescence intensity (MFI) of CD11b in female ($n = 12$) and male ($n = 25$) PMNs showed no significant change ($p = 0.142$). After resting for two hours at 37°C , male ($n = 14$) PMNs showed an increased MFI (CD11b) compared to point zero and in comparison to female PMNs ($n = 24$; see Fig. 13).

3.3.2. Results of CD62L

Directly after isolation (= without resting) MFI(CD62L) of female ($n = 12$) and male ($n = 25$) PMNs showed no difference ($p = 0.142$). After resting for two hours, female ($n = 24$) and male ($n = 14$) PMNs showed

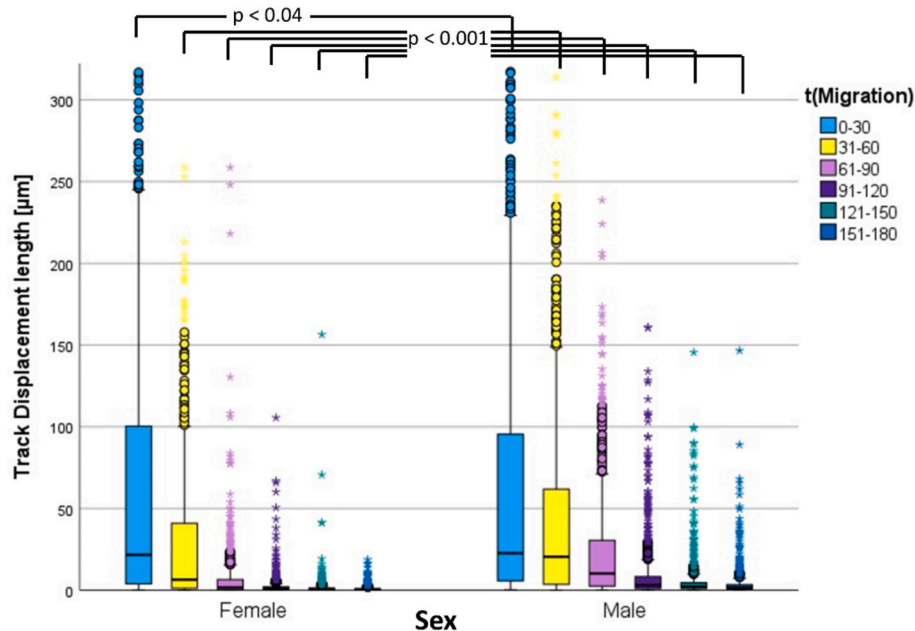


Fig. 8. TrackDisplacementLength was determined with live-cell imaging. Data are shown as median with interquartile range from $n = 12$ experiments. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 3
Overview of medians and interquartile ranges (round brackets) of TrackDisplacementLength (TDL) of the different sexes (individual cell number (n) in square brackets) in determined 30 min time slots, which could be observed by means of live-cell imaging.

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female TDL	21.7 µm (96.6 µm) [$n = 725$]	6.4 µm (40.0 µm) [$n = 687$]	1.6 µm (6.1 µm) [$n = 743$]	0.9 µm (1.5 µm) [$n = 734$]	0.6 µm (1.0 µm) [$n = 752$]	0.4 µm (0.7 µm) [$n = 663$]
	22.7 µm (89.7 µm) [$n = 930$]	20.4 µm (68.3 µm) [$n = 1067$]	10.3 µm (28.0 µm) [$n = 1044$]	3.1 µm (7.1 µm) [$n = 1029$]	2.3 µm (3.5 µm) [$n = 797$]	1.6 µm (2.7 µm) [$n = 778$]
Male TDL						

decreased MFI(CD62L) compared to point zero ($p < 0.001$), but no difference ($p = 0.471$; $p = 0.463$) could be determined between the sexes ($n = 24$; see Fig. 14).

3.3.3. Results of CD66b

Directly after isolation (= without resting) MFI (CD66b) of female ($n = 8$) and male ($n = 22$) PMNs showed no significant change ($p = 0.277$). After resting for two hours, male ($n = 14$) PMNs showed an increased MFI (CD66b) compared to point zero and in comparison to female PMNs ($n = 23$; see Fig. 15).

3.3.4. Results of Rhodamine 123 testing

Both with ($p = 0.156$) and without ($p = 0.189$) resting, no differences of the neutrophil oxidative burst could be determined by rhodamine 123 testing in the flow cytometry between the sexes.

4. Discussion

Between female and male PMNs we observed a different migration behavior and a different surface epitope expression, but no different immune effects. Given the context of sex differences in immune responses, this different behavior is remarkable. For this reason, our observations are examined alongside earlier findings in the following sections.

4.1. Different migration behavior

In our chemotaxis experiments, male PMNs migrated further distances and moved farther from their starting point. However, at the beginning of the live cell imaging, female PMNs migrated in a more targeted way than male PMNs. This changed towards the middle of the observation period, when male PMNs started to migrate more targeted, maintaining straightness towards the end despite very little migration overall in the end of our experiments.

Previous research suggests that sex hormones have a greater impact on the chemotactic migration of PMNs than genetic factors. Specifically, female hormones such as estrogen and progesterone have been found to influence the chemotactic behavior of PMNs (Bouman et al., 2005; Miyagi et al., 1992). More specifically, Jaillon et al. reported that progesterone increased the chemotaxis of PMNs in response to fMLP, while estradiol decreased it. Conversely, testosterone did not demonstrate any significant direct effect on neutrophil chemotaxis (Jaillon et al., 2019). In our experiments, it could not be ruled out that residual traces of sex hormones could be present in the final experimental setup. However, due to the complex isolation steps this seems unlikely. Nevertheless, the male and female cells may have already been primed when they came into contact with sex hormones in vivo prior to isolation.

However, evidence suggests that the chemotaxis of female and male-derived PMNs is different. Relatively little is known about chemorepulsion, where cells move away from a repellent signal. In 2022 it was

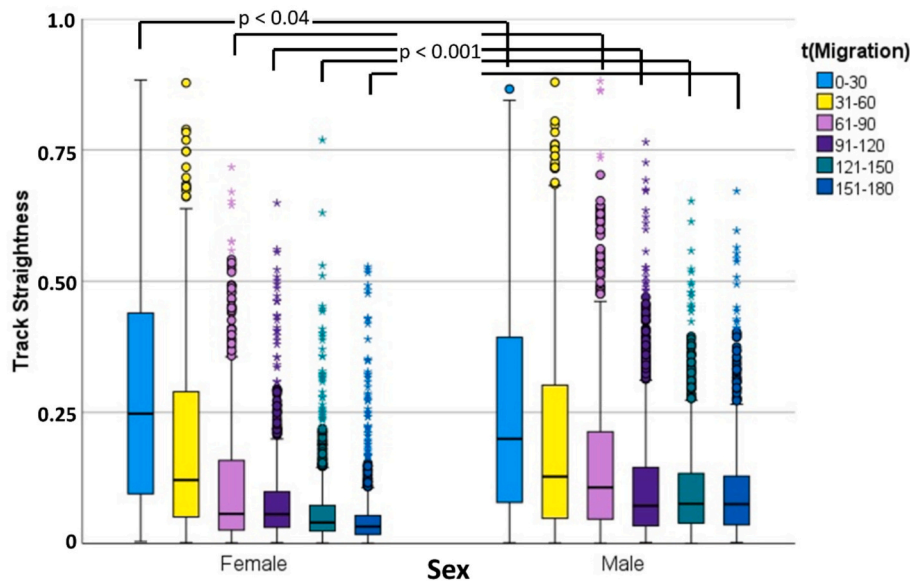


Fig. 9. TrackStraightness was determined with live-cell imaging. Data are shown as median with interquartile range from $n = 12$ experiments. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 4
Overview of medians and interquartile ranges of TrackStraightness (TS) of the different sexes in determined 30 min time slots, which could be observed by means of live-cell imaging (individual cell number (n) in brackets).

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female TS	0.25 (0.34) [n = 725]	0.12 (0.24) [n = 687]	0.06 (0.13) [n = 743]	0.06 (0.07) [n = 734]	0.04 (0.05) [n = 752]	0.03 (0.04) [n = 663]
Male TS	0.20 (0.31) [n = 930]	0.13 (0.26) [n = 1067]	0.11 (0.17) [n = 1044]	0.07 (0.11) [n = 1029]	0.08 (0.09) [n = 797]	0.08 (0.09) [n = 778]

reported that, in the presence of a chemorepellent, female PMNs exhibited a lower percentage of repelled cells, greater persistence in their movement, increased adhesion, and decreased actin polymerization compared to male PMNs (Consalvo et al., 2022).

The differences in our observations could rather be explained by differences in susceptibility to fMLP. Despite no studies can be found in the literature, which directly investigate a possible different nature of the fMLP receptor in men and women, Aomatsu et al. described a different response of male and female PMNs to stimulation with lipopolysaccharides. Their findings indicated that male PMNs show greater responsiveness to LPS stimulation (Aomatsu et al., 2013).

PMNs were outlined as heterogeneous in terms of sensitivity to fMLP. Even though Fletcher and Seligman characterized age as a major influencing factor, they also found differences in sensitivity to fMLP in men and women. Their data indicated that PMNs from healthy adults demonstrate a heterogeneous membrane potential response to the fMLP that correlated with the cells' oxidative responsiveness and that inter-subject differences could be detected. In addition, the proportion of responsive PMNs increased with increasing age in women (Fletcher and Seligmann, 1986).

In summary, our data supports, that female and male PMNs seem to be different in terms of chemotaxis. But the phenomenon of PMN sexual dimorphism extends beyond this. The subsequent sections will address further aspects in greater detail.

4.2. Different surface expression

Differences in the expression of neutrophil surface markers could be determined. Indeed there were no differences directly after isolation,

however, after two hours resting at body temperature CD11b and CD66b expression was significantly greater in male than female PMNs, while CD62L expression did not differ. Our observations support the theory that although PMNs do not exhibit any sex-specific differences from their origin, they do have different properties when primed.

The time of resting was set to two hours because this time is usually corresponding to an apheresis procedure for production of a granulocyte transfusion concentrate, which patients with neutropenia or granulocyte dysfunction may require for adequate immune restoration (Doblinger et al., 2019). Typical recipients of such granulocyte transfusions are patients with neutropenia suffering from progressive infection despite maximum antibiotic therapy (Strauss, 2015; Dullinger et al., 2017).

As our results indicate, such resting (even when physiological temperatures prevailed) could lead to the activation of PMNs (as indicated by increased CD11b and CD66b expression). Thereby, the priming effect was significantly greater in male than that in female neutrophils (Aomatsu et al., 2013).

Our findings align with earlier observations that neutrophil activation, as reflected by CD11b expression and respiratory burst activity, was increased to a greater extent in male rats than in female rats after trauma, hemorrhagic shock or burn injury. Thereby, testosterone appeared to potentiate neutrophil activation, because castration reduced neutrophil activation, whereas ovariectomy had little effect (Deitch et al., 2006). Slightly contradictory are observations of Blazkova et al., who found reduced levels of cell surface maturation markers (CD11c and CD16) in males compared to females. However, they also identified an neutrophil signature (ILNS), including genes encoding for granule-containing proteins, to be upregulated 3-fold in healthy males compared with females (Blazkova et al., 2017). Consequently, sex

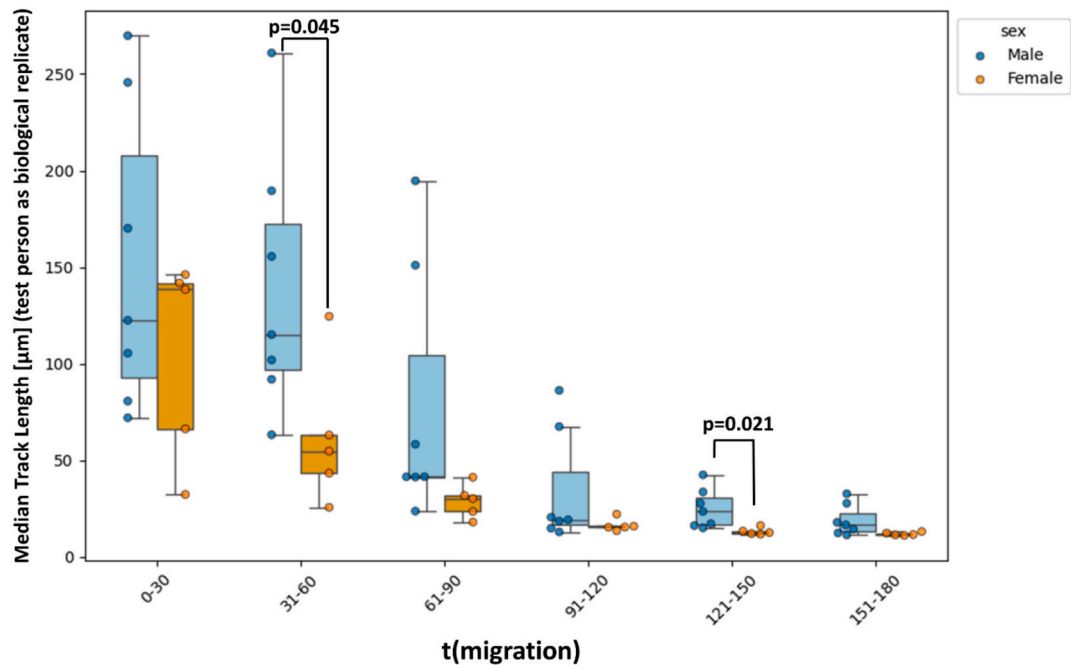


Fig. 10. Overview of migration distances of PMN (TrackLength [μm]), split in observation periods of 30 min, and sex. The single cells of one test person and experiment were combined (median TL) and then further analyzed. Overall migration decreased towards the end of the observation period. The grouped box plot presents the results of *n* = 12 experiments (female: *n* = 5, male: *n* = 7), using medians with interquartil range. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 5
Overview of means and standard deviations [μm] of the medians of parameter migration lengths (TrackLength TL) combined of each test person separated in the different sexes (female: *n* = 5; male *n* = 7) in determined 30 min time slots which could be observed by means of live-cell imaging.

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female mean TL [μm]	104.9 ± 52.4	62.2 ± 37.5	28.8 ± 8.8	16.4 ± 3.3	13.1 ± 1.8	11.8 ± 0.8
Male mean TL [μm]	152.3 ± 79.1	139.7 ± 67.8	78.8 ± 66.2	34.2 ± 29.7	25.0 ± 10.2	18.9 ± 8.1

hormones appear to play a minor role in influencing neutrophil surfaces. As described by Molloy et al. physiologic doses of estradiol and progesterone caused no alterations in neutrophil CD11 (Molloy et al., 2003).

4.3. Influence of sex differences on adverse effects of blood component transfusion

Our results show a certain priming effect due to the storage of PMNs, which is sex dependent and important for any form of transfusion product containing PMNs, but especially for granulocyte transfusions administered in severely neutropenic patients to prevent or treat infections (Strauss, 2015). In this context, it should be kept in mind that TRALI may also develop following transfusion of PMN concentrates. There are rare cases, where preexisting anti-HNA against transfused donor neutrophil antigens in recipients of PMN concentrates cause TRALI by a reverse mechanism (Basu et al., 2022). Nevertheless, the majority of adverse transfusion cases reported are due to transfusion of plasma rich blood components containing anti-HNA against recipient’s cells (Basu et al., 2022). These anti-HNA are found mainly in blood from multiparous women as they have become sensitized during pregnancy (Peters et al., 2015). Thereby, only small amounts of transfusions are sufficient to trigger a TRALI. Aggregating anti-HNA, in particular, have the potential to induce possibly fatal TRALI, even when only a small amount of plasma, like that in red blood cell (RBC) concentrates, is transfused (Schönbacher et al., 2021).

Binding of these anti-HNA to their cognate antigens (including CD11b) causes PMN activation. These activated PMNs sequester in the lungs, causing endothelial damage, capillary leakage and pulmonary edema (van Stein et al., 2016; Browne et al., 2021). We showed that a storage of blood products (only two hours) can affect neutrophil surfaces in a sex-differentiated manner. In this context, Khan et al. described that soluble ligands against neutrophil surface antigens accumulate during blood component storage, have the capacity to activate adherent PMNs, causing endothelial damage and possibly TRALI in predisposed patients (Khan et al., 2006). From these two aspects, we support Stein et al.’s statement that older blood products would therefore be more prone to cause TRALI (van Stein et al., 2016). Nevertheless, the observed changes in the surface epitopes observed in our study could also be due to neutrophil aging, because PMNs undergo rapid aging (Chen et al., 2025). Thus, there have been several studies in the past that observed an activating effect on PMNs when stored, indicated similarly to our data by increased CD11b and decreased CD62L after 24 h storage (Loi et al., 2018). Moreover, Rimboeck et al. observed that PMN cells migrate up to 150 h without activation (e.g. by centrifugation or chemoattractants). Therefore two hours of resting seem unlikely to cause “aging” (Rimboeck et al., 2023). It was previously assumed that “PMN aging” by storage of PMNs, was associated with TRALI. However, however, not the storage time of the transfusion product led to TRALI, but the amount of transfused proinflammatory activity (Loi et al., 2018). During storage of transfusion products (not only PMN concentrate, but also red blood cell or

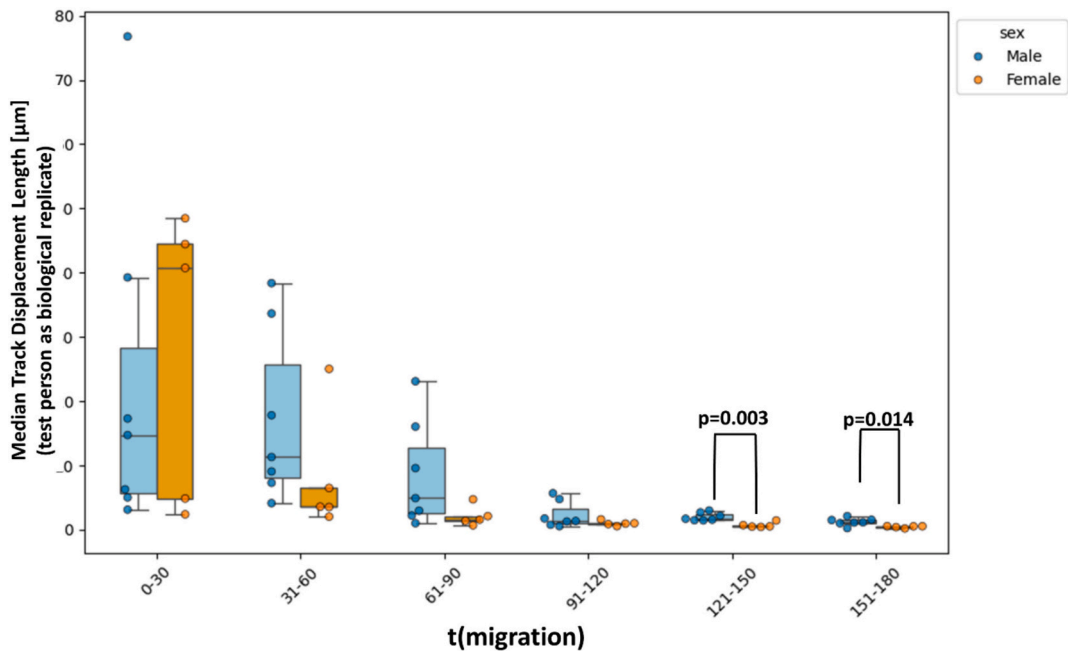


Fig. 11. Overview of migrated euclidean distances of PMNs (TrackDisplacementLength [μm]), split in observation periods of 30 min, and sex. The single cells of one test person and experiment were combined (median TDL) and then further analyzed. The grouped box plot presents the results of $n = 12$ experiments (female: $n = 5$, male: $n = 7$), using medians with interquartil range. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 6
Overview of means and standard deviations [μm] of the medians of migrated euclidean distances (TrackDisplacementLength TDL) combined of each test person separated in the different sexes (female: $n = 5$; male $n = 7$) in determined 30 min time slots which could be observed by means of live-cell imaging.

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female mean TDL [μm]	28.2 ± 22.6	8.1 ± 9.6	2.1 ± 1.6	1.0 ± 0.4	0.7 ± 0.4	0.5 ± 0.1
Male mean TDL [μm]	23.2 ± 26.2	17.4 ± 13.5	8.6 ± 8.3	2.3 ± 2.0	2.0 ± 0.6	1.2 ± 0.6

platelet concentrates), substances (such as non-polar lipids or platelet-derived microparticles) can accumulate which can trigger priming of PMNs and thus be responsible for TRALI (Silliman et al., 2011; Xie et al., 2014). The isolation method (density gradient centrifugation) also influences the PMNs with regard to activation and priming (Kr mer et al., 2023). The observed activation of the examined surface epitopes CD11b, CD66b and CD62L in our study is therefore more likely due to a priming effect than due to PMN aging. Nevertheless, the difference between female and male in this priming process is remarkable.

4.4. No difference in neutrophil immune functions

There was no difference of the immune effects when observing PMNs by live cell imaging. Similarly, no differences in the oxidative burst could be detected in flow cytometry. This results contradict earlier observations by Blazkova et al. who characterized circulating male PMNs with reduced NET formation at baseline and upon bacterial and auto-immune stimuli compared with female PMNs (Blazkova et al., 2017). Moreover, Jaillon et al. reported heightened PMN activation in males during noninfectious inflammatory conditions, including burn injuries, trauma, and hemorrhagic shock, which could contribute to increased tissue damage. The production of reactive oxygen species (ROS) by PMNs was found to be more pronounced in male rats than in proestrus females, and neutrophil activation was significantly diminished following castration. Consequently, it was suggested that sex hormones play a regulatory role in the generation of reactive oxygen intermediates by PMNs (Jaillon et al., 2019). However, this issue remains controversial. PMN free radical production has been shown to be increased (Molloy et al., 2003 (Molloy et al., 2003)), decreased (Bekesi et al., 2000

(B k si et al., 2000)) or not affected (Cassidy, 2003 (Cassidy, 2003)) by estrogen or progesterone incubations in vitro (Bouman et al., 2005).

Nevertheless studies in PMNs from subjects with Klinefelter syndrome (karyotype: 47, XXY) and prepubescent children indicate that sex differences in neutrophil biology are not explained by X chromosome gene dosage and appear to be driven by sex hormones (Gupta et al., 2020). Thus, there are consistent reports in the literature about physiologic doses of female sex hormones estradiol and progesterone affecting neutrophil lifespan and causing a delay in spontaneous apoptosis in both men and women (Jaillon et al., 2019; Molloy et al., 2003). Moreover, estrogen has been shown to be implicated in the formation of NETs (Shepherd et al., 2020). Additionally, Lu et al. stated, that the regulation of chromatin metabolism is a hallmark of neutrophil aging and a key aspect of sex-dimorphic regulation (Lu et al., 2021). Since our experiments were designed without sex hormones, this would explain the lack of different immune effects in our results.

However, there is evidence that female and male PMNs act differently in terms of their immune functions. Klein et al. reportet that the phagocytic activity of PMNs is higher in females than in males (Klein and Flanagan, 2016). According to Pace et al., PMNs from male animals produced less pro-inflammatory cytokines after bacteria-induced prostate inflammation (Pace and Werz, 2020). During noninfectious inflammatory states (such as burn injury, trauma, or haemorrhagic shock) increased PMN activation in male may lead to exacerbated tissue injury (Jaillon et al., 2019). Moreover, in two different trauma models (i.e., thermal injury and traumahaemorrhagic shock), activation of PMN was stronger in male rats compared with proestrus females and castration abrogated PMN activation (Jaillon et al., 2019; Deitch et al., 2006). The discrepancy regarding sexual dimorphism in the PMN immune response

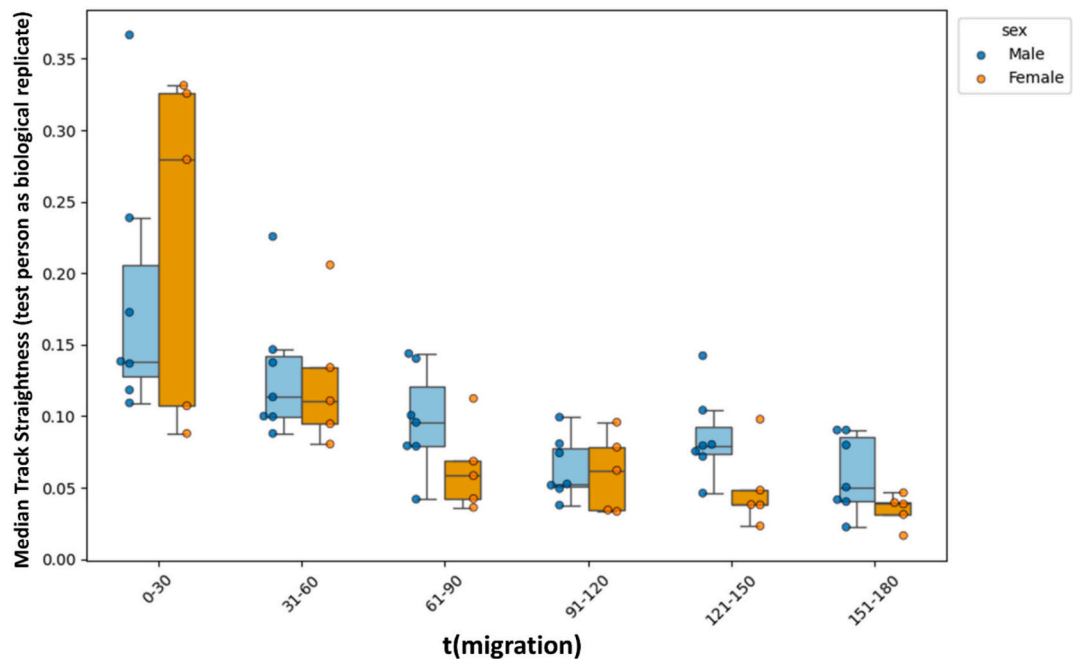


Fig. 12. Overview of the results of parameter track straightness (TS), split in observation periods of 30 min, and sex. The single cells of one test person and experiment were combined (median TS) and then further analyzed. The grouped box plot presents the results of $n = 12$ experiments (female: $n = 5$, male: $n = 7$), using medians with interquartile range. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

Table 7
Overview of means and standard deviations [μm] of the medians of parameter track straightness (TS) combined of each test person separated in the different sexes (female: $n = 5$; male $n = 7$) in determined 30 min time slots which could be observed by means of live-cell imaging.

Time [min]	0–30	31–60	61–90	91–120	121–150	151–180
Female mean TS	0.23 ± 0.12	0.13 ± 0.05	0.06 ± 0.03	0.06 ± 0.03	0.05 ± 0.09	0.03 ± 0.01
Male mean TS	0.18 ± 0.09	0.13 ± 0.05	0.10 ± 0.04	0.06 ± 0.03	0.09 ± 0.03	0.06 ± 0.01

Table 8
Overview of immune effect parameters determined by live-cell imaging displayed by mean $\pm$ standard deviation and sample number (n) in round brackets.

Parameter	T_{maxROS} ($n = 12$)	$ET_{50}\text{MPO}$ ($n = 9$)	$ET_{50}\text{NETosis}$ ($n = 12$)
Female [min]	$144.1 (\pm 17.1)$ ($n = 5$)	$169.5 (\pm 50.6)$ ($n = 4$)	$267.5 (\pm 40.3)$ ($n = 5$)
Male [min]	$152.1 (\pm 44.7)$ ($n = 7$)	$233.9 (\pm 88.6)$ ($n = 5$)	$222.5 (\pm 48.4)$ ($n = 7$)

between previous literature and our data underscores the necessity for further research to elucidate these differences comprehensively.

4.5. Sexual differences in sepsis

Although there is some debate regarding sex-specific responses in sepsis patients, it has been suggested that sex significantly influences post-injury pathogenesis and is associated with differential susceptibility to sepsis, with a higher prevalence observed in males compared to females. A differential immune response regarding the sexes was observed during sepsis, characterized by a distinct profile of pro- and anti-inflammatory cytokines (Vázquez-Martínez et al., 2018). The observed immune response variations in sepsis suggest that females exhibit an initial, regulated pro-inflammatory response that impedes infection. This is followed by an anti-inflammatory response that prevents systemic injury. Conversely, males exhibit a protracted and sustained pro-inflammatory response that ultimately is likely to result in systemic damage (Vázquez-Martínez et al., 2018; Adrie et al., 2007; Bindl et al., 2003).

Our in vitro data can be linked to these clinical observations, which might contribute for understanding sexual difference in sepsis. Female

PMNs demonstrated more directed movement in the beginning of our experiments which might be one of the reasons why women initially show a regulated pro-inflammatory response that hinders infection (Vázquez-Martínez et al., 2018; Adrie et al., 2007). Male PMNs, in contrast, migrated further distances overall and progressively displayed increased targeted migration. Maintaining a higher degree of directional straightness towards the end in combination higher levels of activation markers (CD11b and CD66b) following a two hour resting period could be one reason why men display a late and persistent pro-inflammatory response that leads to systemic damage (Vázquez-Martínez et al., 2018; Bindl et al., 2003). In addition to our migratory data, the presence of sexual dimorphism in severe sepsis in both prepubertal children and elderly individuals suggests that genetic and environmental factors both contribute to this gender bias (Vázquez-Martínez et al., 2018).

4.6. Different migration analysis approaches

When performing live cell imaging and single-cell analysis, researchers are confronted with the question how to analyze the data best. In this manuscript, we also wanted to draw attention to the essential question whether to consider each PMN cell on its own (i.e. as a single

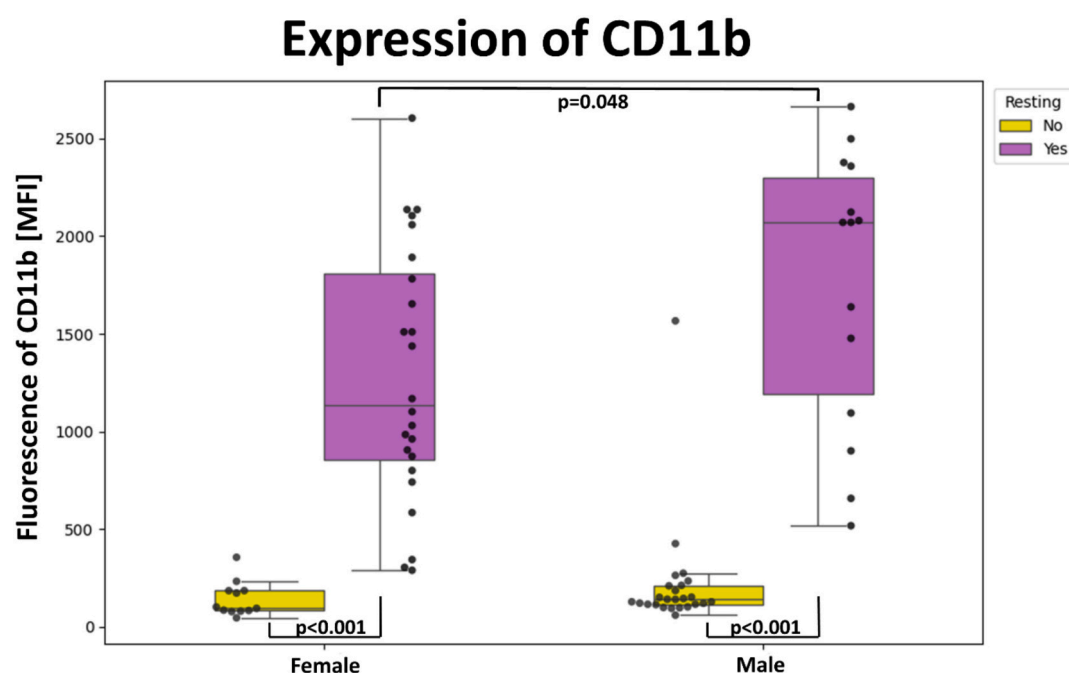


Fig. 13. The mean fluorescence intensity (MFI) of CD11b-PE on male and female PMNs (y-axis) is shown. A distinction is made between female and male PMNs and by resting time of 2 h at 37 °C. Significant differences were observed between male and female cells after 2 h resting and between cells of the same sex with and without resting. Statistical outliers are represented as circles.

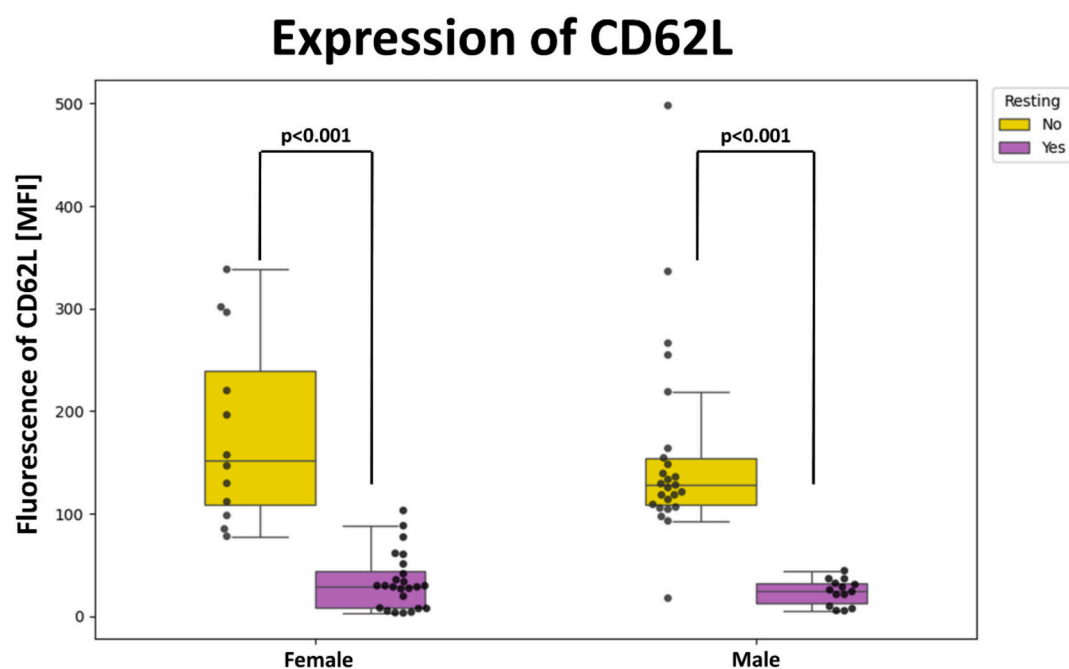


Fig. 14. The mean fluorescence intensity (MFI) of CD62L-FITC on male and female PMNs (y-axis) is shown. A distinction is made between female and male PMNs and by resting time of 2 h at 37 °C. Significant differences were observed in cells between cells of the same sex with and without resting, but no difference could be determined between the sexes. Statistical outliers are represented as circles, and extreme values are depicted as asterisks.

unit), or to consider the PMN cells of one single donor as a single unit, aggregating the individual cells to the level of the donor. This also includes the question of which difference is greater: the difference between the individual cells or the inter-individual difference between the donors. As our sample size is relatively small, we ensured that no donor was duplicated. This was to prevent technical replicates (i.e. multiple neutrophil cells from the same donor) from being represented as biological replicates, which would have posed a problem in terms of

statistical independence. Indeed, when cells come from the same donor, the independence assumption of every statistical analysis would be violated. However, to ensure proper representation of the data, we performed an additional analysis where the data of each donor were summarized as median (see section 3.1.2).

Nevertheless, we support the hypothesis that each PMN cell adapts individually to its environment, and can therefore be regarded as a biological unit. This is the rationale behind the decision to conduct the

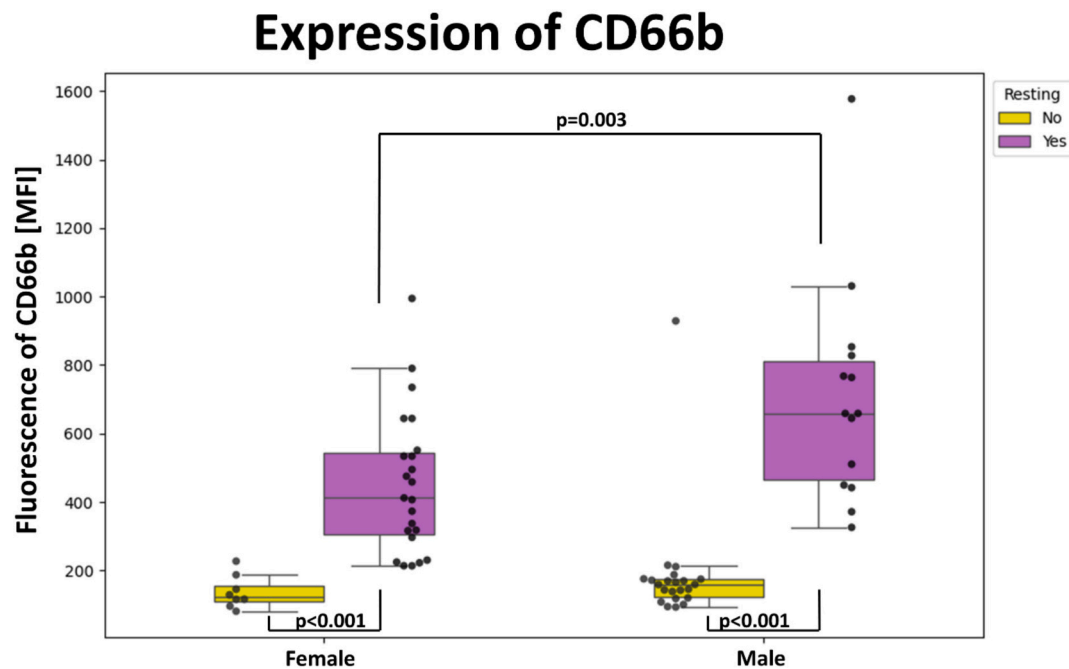


Fig. 15. The mean fluorescence (MFI) of CD66b-APC on male and female PMNs (y-axis) is shown. A distinction is made between female and male PMNs and by resting time of 2 h at 37 °C. Significant differences were observed between male and female cells after 2 h resting and between cells of the same sex with and without resting. Statistical outliers are represented as circles.

primary analysis at the individual cell level, which is a common approach to analyzing LCI experiments for the past decade not only by our research group (Doblinger et al., 2019; Weckmann et al., 2017). Moreover, the rationale of single cell focused analysis is supported by gene analysis observation. In 2021, Grieshaber-Bouyer et al. discovered through genetic analyses that PMNs represent a single developmental continuum. This developmental process is characterized by continual evolution in gene expression from prePMNs through to mature circulating PMNs. Furthermore, they demonstrated how this transcriptional evolution continues during the process of recruitment to inflamed sites, with variations in factors including the site and the stimulus (Grieshaber-Bouyer et al., 2021).

However, it is acknowledged that this approach is not without its challenges. To avoid confounding technical and biological variation (which would have invalidated our statistical analyses), we additionally performed all statistical tests using one summary value (median) per donor. Thereby we aimed to removing the caveat of pseudoreplication to make sure conclusions remain significant and valid. We saw that due to the different analysis, the central tendencies of the data did not differ fundamentally between the two analysis methods. With the summarized to donor level method, statistical significance was only missed in individual time slots due to the reduced number of cases, which would mean an increased number of cases would be needed for significance.

4.7. Limitations of the in vitro models and methods

As stated by Elghetany et al., the selection of anticoagulants utilized in blood collection tubes may influence the specific functions of PMNs (Elghetany and Davis, 2005). In the experiments conducted for this research, whole blood was collected using lithium heparin blood collection tubes, as the heparin anticoagulant was identified as the most appropriate agent for executing functional assays of PMNs, according to a study by Hodge et al. (Hodge et al., 1999). PMNs exhibit a notable susceptibility to mechanical stress, particularly during processes such as centrifugation. The elevated gravitational forces experienced can adversely affect their structural integrity and functional capabilities, potentially resulting in their activation (Hundhammer et al., 2022). The

benefits of isolating PMNs from whole blood through the method of physical density gradient centrifugation, specifically utilizing the leuco/lymphospin technique, are predominantly characterized by a minimal number of washing procedures and a significant level of purity of the resultant cells. Elghetany et al. highlighted this high purity as beneficial for mitigating background signals when employing fluorescently labeled antibodies in the experimental framework, as was conducted in our investigation (Elghetany and Davis, 2005; Kraus et al., 2024b).

The μ -slide chemotaxis chambers® provided by IBIDI, utilized in this study, allow for the embedding of cells within a matrix, creating a three-dimensional environment that mimics the extracellular matrix found in human tissues. As a result, this model is particularly effective for replicating in vivo conditions of the interstitial space. While the μ -slide chambers maintained consistent testing conditions for extended durations, they fundamentally operated as a static system (Kraus et al., 2021). An interstitial flow, which corresponds to the mechanical changes in human tissue caused by blood flow, could not be created. While the PMNs embedded within a gel matrix experienced physical shear forces, these forces were markedly different from the physiological variations typically encountered (Kraus et al., 2021). Nevertheless, it should be noted that the methods used were appropriate for performing functional testing of PMNs at examining sex differences (Kraus and Gruber, 2021b).

5. Conclusion

The results of this study indicates distinct functional variances between male and female PMNs. Male PMNs migrated further distances while female PMNs showed more targeted movement in our chemotaxis experiments. Nevertheless, differences regarding immune effects were not detected. Primarily, there were no sex-specific differences in the expression of neutrophil surface markers directly after isolation. However, after two hours resting at body temperature their expression significantly increased (CD62L, CD11b and CD66b) and showed greater levels in male than in female PMNs (CD11b and CD66b). This priming process might be taken into account when transfusing neutrophil concentrates.

For future studies with PMNs, it is therefore important to select a balanced patient/subject collective (preferably age- and sex-matched) in order to be able to generate convincing results. Our data should also encourage conduction of further studies in transfusion medicine to investigate sex-dependent responses to transfusion products (especially PMN concentrates) in order to confirm the impact on patient care.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We would like to thank Ruth Späth, Regina Lindner, and Gabriele Bollwein (Department of Anesthesiology, University Hospital Regensburg) for their preparing and summarizing of the data sets and for their help in conducting the experiments. We are indebted to Dr. med. dent. Veronika Kraus for her graphical support.

Statements

All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate local institutional committee of the University of Regensburg (vote numbers 16-101-0322, 18-1210-10 and 18-1025-101). The privacy rights of human test persons have been protected and informed consent was obtained prior to their participation in the study at any time. Data availability is limited due to privacy restrictions and can be provided upon request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.imbio.2025.153128>.

Data availability

Data will be made available on request.

References

- Adrie, C., Azoulay, E., Francois, A., Clec'h, C., Darques, L., Schwebel, C., et al., 2007. Influence of gender on the outcome of severe sepsis: a reappraisal. *Chest* 132 (6), 1786–1793.
- Aomatsu, M., Kato, T., Kasahara, E., Kitagawa, S., 2013. Gender difference in tumor necrosis factor- α production in human neutrophils stimulated by lipopolysaccharide and interferon- γ . *Biochem. Biophys. Res. Commun.* 441 (1), 220–225.
- Basu, D., Basu, S., Datta, S.S., 2022. Are we missing reverse TRALI? - a real world experience from a tertiary care oncology Centre in India. *Transfus. Apher. Sci.* 61 (6), 103466.
- Békési, G., Kakucs, R., Váró, S., Rácz, K., Sprintz, D., Fehér, J., et al., 2000. In vitro effects of different steroid hormones on superoxide anion production of human neutrophil granulocytes. *Steroids* 65 (12), 889–894.
- Bindl, L., Buderus, S., Dahlem, P., Demirakca, S., Goldner, M., Huth, R., et al., 2003. Gender-based differences in children with sepsis and ARDS: the ESPNIC ARDS Database Group. *Intensive Care Med.* 29 (10), 1770–1773.
- Blazkova, J., Gupta, S., Liu, Y., Gaudilliere, B., Ganio, E.A., Bolen, C.R., et al., 2017. Multicenter Systems Analysis of Human Blood reveals Immature Neutrophils in Males and during Pregnancy. *J. Immunol.* 198 (6), 2479–2488.
- Bouman, A., Heineman, M.J., Faas, M.M., 2005. Sex hormones and the immune response in humans. *Hum. Reprod. Update* 11 (4), 411–423.
- Browne, T., Dearman, R.J., Poles, A., 2021. Human neutrophil antigens: Nature, clinical significance and detection. *Int. J. Immunogenet.* 48 (2), 145–156.
- Cassidy, R.A., 2003. Influence of steroids on oxidant generation in activated human granulocytes and mononuclear leukocytes. *Shock* 20 (1), 85–90.
- Chen, T., Ren, Q., Ma, F., 2025. New insights into constitutive neutrophil death. *Cell Death Discov* 11 (1), 6.
- Chuang, K.-H., Altuwajiri, S., Li, G., Lai, J.-J., Chu, C.-Y., Lai, K.-P., et al., 2009. Neutropenia with impaired host defense against microbial infection in mice lacking androgen receptor. *J. Exp. Med.* 206 (5), 1181–1199.
- Consalvo, K.M., Kirolos, S.A., Sestak, C.E., Gomer, R.H., 2022. Sex-based differences in Human Neutrophil Chemorepulsion. *J. Immunol.* 209 (2), 354–367.
- Deitch, E.A., Ananthakrishnan, P., Cohen, D.B., Da Xu, Z., Feketeova, E., Hauser, C.J., 2006. Neutrophil activation is modulated by sex hormones after trauma-hemorrhagic shock and burn injuries. *Am. J. Physiol. Heart Circ. Physiol.* 291 (3), H1456–H1465.
- Doblinger, N., Bredthauer, A., Mohrez, M., Hähnel, V., Graf, B., Gruber, M., et al., 2019. Impact of hydroxyethyl starch and modified fluid gelatin on granulocyte phenotype and function. *Transfusion* 59 (6), 2121–2130.
- Dullinger, K., Pamler, I., Brosig, A., Mohrez, M., Hähnel, V., Offner, R., et al., 2017. Granulocytapheresis with modified fluid gelatin versus high-molecular-weight hydroxyethyl starch: a matched-pair analysis. *Transfusion* 57 (2), 397–403.
- Elghetany, M.T., Davis, B.H., 2005. Impact of preanalytical variables on granulocytic surface antigen expression: a review. *Cytometry B Clin. Cytom.* 65 (1), 1–5.
- Fletcher, M.P., Seligmann, B.E., 1986. PMN heterogeneity: long-term stability of fluorescent membrane potential responses to the chemoattractant N-formyl-methionyl-leucyl-phenylalanine in healthy adults and correlation with respiratory burst activity. *Blood* 68 (3), 611–618.
- Grieshaber-Bouyer, R., Radtke, F.A., Cunin, P., Stifano, G., Levescot, A., Vijaykumar, B., et al., 2021. The neutrotome transcriptional signature defines a single continuum of neutrophils across biological compartments. *Nat. Commun.* 12 (1), 2856.
- Gupta, S., Nakabo, S., Blanco, L.P., O'Neil, L.J., Wigerblad, G., Goel, R.R., et al., 2020. Sex differences in neutrophil biology modulate response to type I interferons and immunometabolism. *Proc. Natl. Acad. Sci. U. S. A.* 117 (28), 16481–16491.
- Hodge, G.L., Flower, R., Han, P., 1999. Optimal storage conditions for preserving granulocyte viability as monitored by Annexin V binding in whole blood. *J. Immunol. Methods* 225 (1–2), 27–38.
- Hundhammer, T., Gruber, M., Wittmann, S., 2022. Paralytic Impact of Centrifugation on Human Neutrophils. *Biomedicines* 10 (11).
- Jaillon, S., Berthenet, K., Garlanda, C., 2019. Sexual Dimorphism in Innate Immunity. *Clin Rev Allergy Immunol* 56 (3), 308–321.
- Khan, S.Y., Kelher, M.R., Heal, J.M., Blumberg, N., Boshkov, L.K., Phipps, R., et al., 2006. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood* 108 (7), 2455–2462.
- Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. *Nat. Rev. Immunol.* 16 (10), 626–638.
- Kraus, R.F., Gruber, M.A., 2021a. Neutrophils-from Bone Marrow to First-Line Defense of the Innate Immune System. *Front. Immunol.* 12, 767175.
- Kraus, R.F., Gruber, M.A., 2021b. Neutrophils-from Bone Marrow to First-Line Defense of the Innate Immune System. *Front. Immunol.* 12, 767175.
- Kraus, R.F., Gruber, M.A., Kieninger, M., 2021. The influence of extracellular tissue on neutrophil function and its possible linkage to inflammatory diseases. *Immun Inflamm Dis* 9 (4), 1237–1251.
- Kraus, R.F., Ott, L., Utpatel, K., Kees, M.G., Gruber, M.A., Bitzinger, D., 2024a. Neutrophils in the Spotlight-an Analysis of Neutrophil Function and Phenotype in ARDS. *Int. J. Mol. Sci.* 25 (23).
- Kraus, R.F., Panter, D., Gruber, M.A., Arndt, S., Unger, P., Pawlik, M.T., et al., 2024b. Effects of pressure, Hypoxia, and Hyperoxia on Neutrophil Granulocytes. *Biomolecules* 14 (10).
- Krémer V, Godon O, Bruhns P, Jönsson F, Chaisemartin L de. Isolation methods determine human neutrophil responses after stimulation. *Front. Immunol.* 2023; 14: S. 1301183.
- Lakschevitz, F.S., Hassanpour, S., Rubin, A., Fine, N., Sun, C., Glogauer, M., 2016. Identification of neutrophil surface marker changes in health and inflammation using high-throughput screening flow cytometry. *Exp. Cell Res.* 342 (2), 200–209.
- Libert, C., Dejager, L., Pinheiro, I., 2010. The X chromosome in immune functions: when a chromosome makes the difference. *Nat. Rev. Immunol.* 10 (8), 594–604.
- Liu, Y., Wang, R., Song, C., Ding, S., Zuo, Y., Yi, K., et al., 2023. Crosstalk between neutrophil extracellular traps and immune regulation: insights into pathobiology and therapeutic implications of transfusion-related acute lung injury. *Front. Immunol.* 14, 1324021.
- Loi, M.M., Kelher, M., Dzieciatkowska, M., Hansen, K.C., Banerjee, A., West, F.B., et al., 2018. A comparison of different methods of red blood cell leukoreduction and additive solutions on the accumulation of neutrophil-priming activity during storage. *Transfusion* 58 (8), 2003–2012.
- Lu, R.J., Taylor, S., Contrepois, K., Kim, M., Bravo, J.I., Ellenberger, M., et al., 2021. Multi-omic profiling of primary mouse neutrophils predicts a pattern of sex and age-related functional regulation. *Nat Aging* 1 (8), 715–733.
- Miyagi, M., Aoyama, H., Morishita, M., Iwamoto, Y., 1992. Effects of sex hormones on chemotaxis of human peripheral polymorphonuclear leukocytes and monocytes. *J. Periodontol.* 63 (1), 28–32.
- Molloy, E.J., O'Neill, A.J., Grantham, J.J., Sheridan-Pereira, M., Fitzpatrick, J.M., Webb, D.W., et al., 2003. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. *Blood* 102 (7), 2653–2659.
- Pace, S., Werz, O., 2020. Impact of Androgens on Inflammation-Related Lipid Mediator Biosynthesis in Innate Immune Cells. *Front. Immunol.* 11, 1356.
- Pai, D., Gruber, M., Pfähler, S.-M., Bredthauer, A., Lehle, K., Trabold, B., 2020. Polymorphonuclear Cell Chemotaxis and Suicidal NETosis: Simultaneous Observation using fMLP, PMA, H7, and Live Cell Imaging. *J. Immunol. Res.* 2020, 1415947.
- Peters, A.L., van Stein, D., Vlaar, A.P.J., 2015. Antibody-mediated transfusion-related acute lung injury: from discovery to prevention. *Br. J. Haematol.* 170 (5), 597–614.
- Rimboeck, J., Gruber, M., Wittmann, S., 2023. Is the In Vitro Observed NETosis the favored Physiological death of Neutrophils or mainly Induced by an Isolation Bias? *Int. J. Mol. Sci.* 24 (8).

- Schönbacher, M., Aichinger, N., Weidner, L., Jungbauer, C., Grabmer, C., Schuha, B., et al., 2021. Leukocyte-Reactive Antibodies in Female Blood Donors: the Austrian experience. *Transfus. Med. Hemother.* 48 (2), 99–108.
- Shepherd, R., Cheung, A.S., Pang, K., Saffery, R., Novakovic, B., 2020. Sexual Dimorphism in Innate Immunity: the Role of sex Hormones and Epigenetics. *Front. Immunol.* 11, 604000.
- Silliman, C.C., Moore, E.E., Kelher, M.R., Khan, S.Y., Gellar, L., Elzi, D.J., 2011. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. *Transfusion* 51 (12), 2549–2554.
- van Stein D, Beckers EAM, Peters AL, Porcelijn L, Middelburg RA, Lardy NM et al. Underdiagnosing of antibody-mediated transfusion-related acute lung injury: evaluation of cellular-based versus bead-based techniques. *Vox Sang.* 2016; 111(1): S. 71–78.
- Strauss, R.G., 2015. Neutrophil/granulocyte transfusions collected from G-CSF + dexamethasone-stimulated donors. *Curr. Opin. Hematol.* 22 (6), 565–567.
- Vázquez-Martínez, E.R., García-Gómez, E., Camacho-Arroyo, I., González-Pedrajo, B., 2018. Sexual dimorphism in bacterial infections. *Biol. Sex Differ.* 9 (1), 27.
- Weckmann, M., Becker, T., Nissen, G., Pech, M., Kopp, M.V., 2017. SiMA: a simplified migration assay for analyzing neutrophil migration. *Cytometry A* 91 (7), 675–685.
- Xie, R.F., Hu, P., Li, W., Ren, Y.N., Yang, J., Yang, Y.M., et al., 2014. The effect of platelet-derived microparticles in stored apheresis platelet concentrates on polymorphonuclear leucocyte respiratory burst. *Vox Sang.* 106 (3), 234–241.