fibromyalgia syndrome. Neutrophils are polymorphonuclear granulocytes that normally exist in circulation to function as primary mediators of rapid innate host defence, releasing extracellular vesicles as functional mobile units. Surprisingly, neutrophils are found in increased circulatory levels and with enhanced chemotactic and microbial properties in fibromyalgia patients. Furthermore, we have recently shown a key role for neutrophils in mediating the spatiotemporal spread of hypersensitivity in a hyperalgesic priming model of chronic widespread pain. Our data also shows a fundamental pro-nociceptive action of neutrophils derived from patients or mice with chronic widespread pain when administered to naïve receptor mice.

**Objectives:** The mechanisms by which neutrophils can sensitise sensory neurons to cause pain signalling is still unclear but is likely linked to a distinct subpopulation of neutrophils characterised by altered surface protein expression. We aim to identify a specific neutrophil subpopulation that sensitises nociceptive neurons either through cell-cell interactions or mediated via neutrophil-derived extracellular vesicles (NDEVs) to produce chronic widespread pain in fibromyalgia syndrome. Methods: To identify a pro-nociceptive neutrophil population, we used neutrophils from fibromyalgia patients and pain free controls, characterising proteomic differences between the cohorts by FACS. Furthermore, we have employed functional assays of neutrophil reactivity, including quantification of reaction oxygen species (ROS) production and neutrophil extracellular trap generation (NETosis) to characterise specific functional differences which might be related to a pro-nociceptive capacity. To assess the role of NDEVs in neutrophils pro-nociceptive actions we have isolated and performed phenotypic characterisation of NDEVs from patient and pain-free controls. Finally, we used primary sensory neuron cultures to assess the effect of neutrophils and NDEVs on neuronal excitability using functional assays (calcium imaging and in vitro electrophysiology) to demonstrate the capacity of neutrophils to sensitize peripheral sensory neurons.

**Results:** Proteomic characterisation of neutrophils derived from patients with fibromyalgia pain vs pain free subjects reveals a distinct population of cells, including altered expression of cell surface markers CD62L and CXCR2. Quantification of ROS generation also reveals changes in cells derived from patients when compared to pain free counterparts, alongside increased NETosis which may reflect specific mechanisms employed by cells to sensitize peripheral neurons following trafficking to DRG. We observe increased NDEV release from patient cells alongside specific phenotypic differences, including an upregulation of surface markers, mirroring what we observe within the parent cells. Moreover, in vitro systems, including electrophysiology recording and calcium imaging reveal increased neuronal responses to both patient cells and patient cell-derived NDEVs.

**Conclusion:** We show a surprising role of neutrophils, a short-lived immune cell, in the aetiology of chronic widespread pain in fibromyalgia syndrome. Our data demonstrates altered proteomic profiles and distinct phenotypic differences in both circulating neutrophils and NDEVs from patients, supporting an immunological basis for chronic widespread pain in fibromyalgia and a novel mechanism of nociceptive pain.

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**Figure 1.**

**PO0256 IDENTIFICATION OF TWO BIOLOGICAL SUBTYPES OF CRPS**

**Keywords:** Pain, Skin

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**Background:** Patients with Complex Regional Pain Syndrome (CRPS) present prolonged and debilitating pain and tissue damage. To date, treatment remains a challenge. Biological phenotyping has not been performed yet, and thus, molecular therapies are missing.

**Objectives:** To determine whether different biological CRPS subtypes exist and to provide a molecular profile of these subtypes.

**Methods:** CRPS patients fulfilling the Budapest criteria were included. Body Perception Disturbance (BPD) and pain scores (0-100) questionnaires were recorded. Skin punch biopsies (3 millimetres) from the affected and non-affected sides (n=6+6) and matching blood samples were taken. Biopsies were split into two for transcriptomics and histological analyses. Blood was passivated to collect plasma after centrifugation and peripheral blood mononuclear cells (PBMCs) with a Ficoll gradient. Total RNA was isolated from skin biopsies and from PBMCs for bulk sequencing. Differentially expressed genes (DEGs) were determined using DESeq2 generalised linear model (p≤0.01, log2 ratio≥0.5). Hierarchical clustering and principal component analysis (PCA) were performed on raw transcriptomic data to identify patient sub-populations. Cell types were approximated in skin and PBMCs with deconvolution analysis using RNA sequencing databases with known cell frequencies (PRESST cohort and immunedeconv, respectively). Twenty pro- and anti-inflammatory plasma cytokines were measured using chemiluminescence-based assays (MDSD U-PLEX Viral Combo 1). Histological slides were scored (0-3) blinded by a dermato-pathologist based on a panel of 10 morphological and cellular characteristics. Protein levels, histological evaluations and demographic data were compared between CRPS clusters using unpaired t-tests.

**Results:** Six women with CRPS 1 of the hand (n=5) and foot (n=1) participated in the study. Mean age was 47.3 ± 14.2 yrs and mean symptom duration was 57.8 ± 44.2 weeks. Skin transcriptomics showed 427 DEGs when comparing affected vs. non-affected samples (Figure 1a). Samples clustered by patient rather than affected/non-affected side (Figure 1b). PCA of only the affected samples displayed two clusters (PC1=65%, PC2=17%) (Figure 1c). Further evaluation of all affected samples by deconvolution analysis suggested two distinct cellular signatures, clustering patients in the same way as the PCA. Keratinocyte-related genes were upregulated in cluster 1 (n=3) and strong fibroblast, microvascular endothelial cell, monocyte and M1 macrophage signatures were found in cluster 2 (n=3) (Figure 1d). Consequently, gene ontology (GO) of each cluster showed enrichment of epithiome development and downregulation of peptide terms in cluster 1 and extracellular matrix and collagen fibril organisation in cluster 2. Histopathological examinations did not show any difference between subtypes. PCA of PBMCs transcriptome validated the same distribution of patients into two clusters (PC1=62%, PC2=16%) (Figure 1e). A total of 2'328 DEGs were found between both clusters, with an overrepresentation of immune and inflammatory response, and neutrophil degranulation and chemotaxis-related terms in cluster 1 (n=2) according to GO. Deconvolution analysis confirmed the presence of a stronger signature of neutrophils and M2 macrophages in cluster 1 than cluster 2. Blood cytokine quantification showed that interleukin-1 receptor antagonist (IL-1RA) levels were 1.9 times higher in cluster 1 than in cluster 2 (n=3+3, p=0.02) (Figure 1f). Consistently, transcriptomic data indicated that cluster 1 had higher expression of IL-1RA encoding gene (IL-1RN) in matching skin (log2 ratio=0.88, FDR=0.06) and PBMCs (log2 ratio=1.1, FDR=0.01), than cluster 2. There was no significant difference in age or symptoms duration between clusters, but pain and BPD questionnaires revealed differences between groups, suggesting that the clusters might be clinically distinguishable (Figure 1g).

**Conclusion:** Our findings suggest the existence of two biological CRPS subtypes. Molecular patterns could facilitate the development of targeted therapies.

**REFERENCES:** NIL.

**Acknowledgements:** NIL.
Background: Chronic pain is highly prevalent, debilitating and lacks effective treatments. Experimental and neuroimaging research demonstrates abnormal central pain processing[1]. However, robust brain-based biomarkers that could inform targeted treatments are lacking. Electroencephalography (EEG) is the optimal tool to investigate dynamic abnormalities in pain processing to reveal underlying mechanisms. Early evidence from EEG studies in Fibromyalgia (FM) indicate potential mechanisms such as thalamocortical dysrhythmia[2], demonstrated by alterations in the Alpha and Theta frequency bands. However, existing studies employ rudimentary analyses failing to account for the multivariate and temporal nature of EEG data. State-of-the-art machine learning (ML) approaches provide unique opportunities to generate a deeper understanding of EEG signatures in chronic pain and identify specific biomarkers that could be used to differentiate mechanistic subtypes.

Objectives: This preliminary work aims to establish whether a state-of-the-art ML classifier can differentiate patients with FM from healthy controls based on their EEG characteristics.

Methods: The dataset used was collected through The VIPA Study (ISRCTN46681140). Patients with FM satisfied the 2016 FM classification criteria. High-density 64-channel EEG data using an electrolyte gel-based active electrode system was collected at rest with participants’ eyes closed over 2 minutes. Individual Fast Fourier Transforms were applied to overlapping time windows to extract EEG frequency band power whilst retaining temporal information. Frequency bands were used as features to train a classification model (definitions of frequency ranges in Table 1). One of the fastest, most accurate state-of-the-art time-series classification ML algorithms (mini-ROCKET) was used via the sktime python toolkit. To obtain unbiased accuracy estimates across all participants, a ‘leave-one-out’ strategy was used. Accuracy of the algorithm was reported; defined by the number of correct predictions divided by the total number of predictions for each frequency band (2-class problem, chance estimates 0.5-0.6 based on p<0.05).

Results: Data from 23 patients with FM (mean age 46 ±14yrs, 87% female) and 14 healthy controls (mean age 71 ±7yrs, 50% female) were analysed. Patients with FM had moderate self-reported pain (5.5 ±2.3 VAS) and disease severity (mean FIQR scores are worse) using the same study app, and passively recorded total time being active.

Conclusion: Preliminary results indicate that machine learning can be successfully used to differentiate patients with Fibromyalgia from healthy controls based on EEG measures of the Alpha and Theta frequency bands. Alterations in Alpha and Theta have been demonstrated in previous non-ML research, indicating potential underlying abnormalities in the interaction between the thalamus and cortex which may be related to central mechanisms underlying chronic pain. Further work is required in larger, matched cohorts to validate these findings, but this early work highlights the future potential of EEG and ML in both understanding brain-based pain mechanisms and using EEG features to differentiate chronic pain subgroups.

REFERENCES:

Table 1. Classifier accuracy across the EEG frequency bands (FM vs Control)

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>Accuracy of mini-ROCKET classifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta (2-4Hz)</td>
<td>0.512 (51.2%)</td>
</tr>
<tr>
<td>Theta (4-8Hz)</td>
<td>0.707 (70.7%)</td>
</tr>
<tr>
<td>Alpha (8-12Hz)</td>
<td>0.707 (70.7%)</td>
</tr>
<tr>
<td>Beta (12-30Hz)</td>
<td>0.634 (63.4%)</td>
</tr>
<tr>
<td>Gamma (30-40Hz)</td>
<td>0.610 (61.0%)</td>
</tr>
</tbody>
</table>

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Keywords: Pain, Imaging, Artificial Intelligence

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Keywords: Pain, Rheumatoid arthritis

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Background: Pain flares in rheumatoid arthritis (RA) often refer to episodes of increased pain severity accompanied by pain impact. Describing the prevalence and predictors of pain flare occurrence is difficult since there are no agreed classification criteria[1]. Patient-generated data collected in real-time with mobile health (mHealth) devices provide an opportunity to identify individual patterns and triggers in pain dynamics over time[2].

Objectives: We aim to characterise pain flares and pre-flare exposures using real-time mHealth data from patients with RA.

Methods: In a 30-day mHealth study[3] we collected daily reports of pain severity on a five-point scale (ranging from none to very severe pain) via a smartphone app to define the onset and ending criteria for three types of pain flares, including 1) above average: pain severity greater than the personal median score, 2) significant change: two-point increase in pain severity from yesterday, and 3) absolute impact: two-point increase in pain severity from yesterday and pain severity greater than three. All pain flare types end when pain severity returns to the personal median score or lower. Exposures of the preceding periods were self-rated sleep quality, mood, anxiety and fatigue (all scales range 1-5, higher scores are worse) using the same study app, and passively recorded total time asleep (hr), sleep efficiency (%), sleep latency (min) and physical activity (min) via a wrist-worn accelerometer. We report the 30-day monthly pain flare rate, the average duration of pain flares and summarise average exposures one-day and three-day before pain flare onset.

Results: We analysed 253 participants who provided at least seven days of data (81.8% females; mean age = 59.9, average years with RA = 12.1). A total of 6,244 daily reports were included in the analysis. Pain flare occurrence decreased when applying more complex definitions. 31% of participants had pain flares under the most stringent definition of absolute impact, with two episodes per month (Table 1). Across all types, 75% of pain flares lasted two days before returning (Median = 1, IQR = 1-2) but could persist up to 11 days (Figure 1). Pre-flare exposures did not differ between pain flare types nor between the preceding periods. Participants reported fair sleep quality (Median = 3), feeling quite happy (Median = 2), not anxious (Median = 1) and mild fatigue (Median = 2) prior to pain flare onset. Objective exposures showed a daily average of 7-hour sleep with 83% efficiency, under 20 minutes to fall asleep, and approximately 50 minutes being active.

Table 1. Pain flare characteristics

<table>
<thead>
<tr>
<th>Type</th>
<th>Number of participants with ≥1 pain flare(s) (%)</th>
<th>Total number of pain flares</th>
<th>Monthly pain flare rate (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above average</td>
<td>224 (88.5)</td>
<td>788</td>
<td>4.3 (2.2)</td>
</tr>
<tr>
<td>Significant change</td>
<td>108 (42.7)</td>
<td>171</td>
<td>2.0 (1.1)</td>
</tr>
<tr>
<td>Absolute impact</td>
<td>78 (30.8)</td>
<td>116</td>
<td>2.0 (1.1)</td>
</tr>
</tbody>
</table>

Disclosures of Interests: None Declared.

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