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Biological and Psychosocial Factors Affecting the Persistence of Pruritus Symptoms – Protocol of Project 4 of the Interdisciplinary SOMACROSS Research Unit (RU 5211)

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Biological and Psychosocial Factors Affecting the Persistence of Pruritus Symptoms – Protocol of Project 4 of the Interdisciplinary SOMACROSS Research Unit (RU 5211)

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ABSTRACT

Introduction: Chronic pruritus (CP) is a symptom of many dermatologic, neurologic, systemic and psychosomatic diseases. CP has a prevalence of ~20% in the general population and is therefore a significant burden on society, but the transition from acute pruritus to CP is not well understood. It probably involves interactions between biological and psychosocial factors and pruritus-specific risk factors as well as mechanisms shared with other persistent somatic symptoms, which are also addressed in other projects and the overarching aims of the SOMACROSS Research Unit (RU).

In this project we aim to identify psychosocial and biological factors, as well as their interactions, which contribute to the persistence of CP with and without immunologic / inflammatory origin, i.e. atopic dermatitis and pruritus on non-inflamed skin. We expect that psychosocial factors relevant to the persistence of symptoms in conditions such as fatigue and pain may also contribute to CP.

Methods and analysis: Three cohorts of 40 patients each with acute exacerbation of atopic dermatitis and chronic atopic dermatitis and 40 CP patients with unaffected skin will be recruited for a comprehensive translational investigation. This will include pruritus-specific and the shared psychosocial assessments of the RU SOMACROSS. Pruritus-specific measures will include questionnaires, neurological parameters (using quantitative sensory testing), cutaneous nerve fibre morphology, skin barrier morphology, epidermal metabolism, and pruritogen blood levels. Within one year, patients and 80 age- and sex-matched healthy controls will be examined at three time points, allowing cross-sectional comparison and a longitudinal investigation of predictive outcome factors in patients under treatment according to existing guidelines.

Ethics and dissemination: The study has been approved by the ethics committees of Hamburg and Münster, Germany. Findings will be disseminated through peer-reviewed publications, scientific conferences, and involvement of relevant stakeholders, patients and the lay public.

Registration: German Register for Clinical Studies, Study-Id: DRKS00026646.

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Key words:

Pruritus; Itch; Persistent Somatic Symptoms; Mechanisms; Risk Factors; Expectations;
Research Unit; Biopsychosocial Models; Prediction Models

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- Chronic pruritus is a symptom of many dermatologic, neurologic, systemic and psychosomatic diseases and has a prevalence of ~20% in the general population. It causes distress and disability and is a significant burden on society. However, the transition from acute pruritus to CP is not well understood up to now.
- The comprehensive approach of this project has not been attempted before: To analyse biomarkers of the skin and blood, the function and anatomical structure of the skin barrier, the peripheral nervous system and its interaction with the central nervous system, as well as psychological and social factors in two key forms of CP (inflammatory and non-inflammatory). In addition, we will follow the course of pruritus over 12 months and relate the progression of the disease to the above parameters in order to identify psychosocial and biological factors that contribute to the persistence of pruritus. We will increase the value of the work by studying three distinct groups of pruritus patients: acute and chronic atopic dermatitis and pruritus on non-lesional skin.
- The crosslink with the other projects of the RU SOMACROSS and the use of one common set of instruments in all projects is a completely new strategy that allows us to compare results between projects and to distinguish between symptom-specific and generic mechanisms.
- The novel techniques available in this project (Atomic Force Microscopy (AFM), Hyperspectral Imaging (HIS) and Multiphoton Tomography equipped with a Fluorescence Lifetime Imaging module (MPT-FLIM) have not been applied to patients with atopic dermatitis and chronic pruritus on non-lesional skin before.
- Despite investigating multiple potential risk factors and mechanisms of the persistence of chronic pruritus, other variables, which are not addressed in this project, might be relevant.

INTRODUCTION

State of the art

Chronic pruritus (CP), also known as chronic itch, is defined as the presence of itching that lasts for 6 weeks or more. CP is a symptom in many dermatologic, systemic, neurologic and psychosomatic diseases. It has a high burden on society, with a prevalence of ~20% in the general population and across various medical specialities.^{1,2} The onset and course of CP can be influenced by different psychosomatic aspects and psychologic comorbidities.^{3,4}

CP therefore is classified as a persistent somatic symptom (PSS) and fits conceptual models of PSS aetiology.⁵ Like other forms of PSS, CP is thought to arise from complex interactions between the brain, body and environment (Figure 1). Thus, this fits well into the interdisciplinary Research Unit SOMACROSS, which has been presented by Löwe et al. ⁶ in this journal.

- Figure 1 approx. here-

Not all of the biological, psychological and social factors and their interactions that promote the transition from acute pruritus to CP are well understood. Persistence of CP is likely to involve pruritus-specific pathophysiological, psychological and social risk factors and mechanisms that are shared with other forms of PSS. A comprehensive model of the aetiology and persistence of CP still needs to be validated.

-Figure 2 approx.here-

Acute pruritus may be triggered by chemical, physical or mechanical stimuli. Pruritus may be induced by pruritogens released from inflammatory cells and keratinocytes, as well as nerve fibres of the peripheral or central nervous system. Cutaneous group C and group A nerve fibres carry corresponding receptors for interleukins (ILs) such as IL31 and IL4, histamine, or neuropeptides such as substance P and somatostatin.^{1,7}

Atopic dermatitis (AD) is the most prevalent pruritic dermatologic disease and the most widely studied model of immunologic and skin-barrier related pruritus mechanisms. AD can occur in acute phases, and chronic phases with persistent pruritus and subacute eczema. At all stages, patients suffer from intense pruritus regardless of the severity of the eczema.⁸ On the other hand, CP may also occur on non-inflamed, non-lesional skin (CPNL), e.g. due to systemic, neurologic or other diseases.

Skin barrier dysfunction is widely considered to be the primary event in AD and is pivotal for pruritus.⁹ The outermost layer of the epidermis (stratum corneum) is the most relevant skin layer for the cutaneous barrier function. In AD skin lesions, the expression of tight junction proteins such as claudin-1 is reduced, and the stratum corneum becomes 'leaky' and shows dramatic morphological and structural changes shown by atomic force microscopy (AFM). Furthermore, the skin barrier is also impaired in clinically non-lesional AD skin. The skin barrier dysfunction, along with a biphasic Th2/Th1 response, the release of pruritogenic cytokines, epidermal hypoinnervation, and neuronal hypersensitivity, are considered essential for the induction and most likely also the persistence of pruritus although the contribution of psychosocial factors is unclear.¹⁰

Neural hypersensitivity of the peripheral and/or central nervous system has been demonstrated in AD and CP of other origin.^{1,4,11}. In this state, nerve fibres are more vulnerable

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to pruritogens, the corresponding receptors / ion channels have a lower activation threshold, and neuromediator production in nerve fibres is enhanced⁴. E.g., cutaneously applied pruritogens evoke pruritus that is more intense and longer lasting than usual (hyperknesis).^{1,12} In contrast, alloknesis describes the induction of pruritus by non-pruritogenic stimuli such as mechanical triggers.

Mechanisms contributing to neuronal hypersensitivity are largely unknown but may include chronic stimulation with inflammatory pruritogens such as IL31 and IL4,⁴ permanent scratching,^{13,14} the depletion of cutaneous Merkel cells,¹⁵ psychological and/or social factors.¹⁶ Furthermore, several previous studies have shown that social and psychological factors are risk factors for an enhanced perception of pruritus (**Figure 2**).

Positive and negative expectations, which can also be induced by corresponding placebo and nocebo instructions, have been shown to influence the intensity of pruritus, scratching behaviour and even the physiologic skin reactions after experimentally induced pruritus in healthy controls^{17,18} and in patients with AD¹⁹. Anxiety, depression²⁰ and personality traits such as neuroticism (i.e., emotional instability with the tendency to experience more negative emotions), low agreeableness^{20,21} and low self-efficacy²² were associated with a stronger perception of pruritus in patients with AD, psoriasis, and in healthy controls. Self-reported pruritus has been repeatedly linked to self-reported perceptions of stress and stressful life events in population samples^{19,22} and also in AD patients.²³⁻²⁶

Biologically, patients with AD may show a response to stress in terms of T-cell and mast cell activation, expression and release of neurogenic inflammatory mediators, and disruption of normal epidermal barrier function.²⁶ Stress has been shown to deteriorate the skin barrier by activating 11beta-Hydroxysteroid Dehydrogenase 1 and the HPA axis.²⁷ The association between stress and pruritus can also be mediated by negative pruritus-associated cognitions and expectations in AD patients (**Figure 2**).²⁵

Conclusion:

Taken together, the results of multiple studies confirm psychological factors to be risk factors for an enhanced perception of pruritus, but the extent to which these factors contribute to the persistence and chronicity of CP remains unclear (**Figure 1**).

Novelty of the study

CP has biological, psychological and social dimensions and the interactions between these factors are poorly understood. Data gathered so far were mostly derived from cross-sectional studies not set out to identify such interactions. We will therefore apply a comprehensive set of instruments to analyse biomarkers of the skin and blood, the function and anatomical structure of the skin barrier, the peripheral nervous system and its interaction with the central nervous system, as well as psychological and social factors in two key forms of CP (inflammatory and non-inflammatory). In addition, we will follow the course of pruritus over 12 months and relate the progression of the disease to the above parameters in order to identify psychosocial and biological factors that contribute to the persistence of symptoms. Such a comprehensive approach has not been attempted before, and we will increase the value of the work by studying three distinct groups (two inflammatory and one non-inflammatory) of pruritus patients. The crosslink with the other projects of the RU SOMACROSS and the use of one common set of instruments in all projects is a completely new strategy that allows us to compare results between projects and to distinguish between symptom-specific and generic

mechanisms. The novel techniques available in this project have not been applied to patients with AD or CPNL before.

Objectives

The overall aim of the project is to identify psychosocial and biological factors (and their interactions) that contribute to the persistence of symptoms in CP. The psychosocial factors relevant to the persistence of symptoms in conditions such as fatigue and pain may also contribute to CP. Therefore, the project has the following main objectives:

1. To characterize psychosocial risk factors for: (i) the persistence of CP in general, and (ii) the persistence of CP of cutaneous inflammatory and non-inflammatory origin in particular.
2. To determine whether there are associations between psychosocial risk factors and specific peripheral and/or central neuronal sensitization patterns in CP.
3. To determine co-variation of modifiable psychosocial risk factors and biological factors (e.g., cellular metabolism and skin barrier function).

Hypothesis:

Psychosocial (personality, stress, expectations, catastrophizing, illness perceptions, anxiety, depression) and biological factors (central sensitization processes, cutaneous neuroanatomy, cellular metabolism, skin barrier function) interact in promoting and predict the persistence of pruritus.

METHODS AND ANALYSIS

Study design and participants

Participants: Patients with AD will be included as a model of cutaneous inflammatory pruritus, whereas patients with CPNL (CP with pruritus on non-lesional skin) will be included as a model of non-inflammatory pruritus. AD starts in childhood and we cannot capture the long-term follow up starting at the onset of the disease, so we will recruit two stages of AD: a group of adult patients (group 1; n = 40) presenting with acute AD (after a manifestation-free interval of at least 6 months), and a group of adult patients (group 2; n = 40) presenting with chronic AD. These groups will be subject to cross-sectional and longitudinal comparisons. We will also recruit CPNL patients (group 3; n = 40) in which CP has typically developed over the course of several years. We will compare the patient groups cross-sectionally with age/sex-matched skin-healthy controls (group 4; n = 80).

- Figure 3 approx.here -

Inclusion criteria:

All projects of RU SOMACROSS share common basic inclusion criteria, i.e.: age ≥18 years, sufficient oral and written German language proficiency to complete self-report questionnaires and interviews and written informed consent. In addition to these common criteria, the project-specific inclusion criteria are listed below.

Group 1: CP in acute AD (n = 40)

→ acute eczema for ≤ 4 weeks at inclusion, no eczema in the previous 6 months

Group 2: CP in chronic AD (n = 40)

→ AD and CP for at least 6 weeks, chronic eczema

Group 3: CP in CPNL (n = 40)

→ CP for at least 6 weeks, clinically no dermatosis, no prurigo

Groups 1–3: pruritus intensity ≥ 5 , worst intensity in last 24 h on numeric rating scale (NRS, range 0 – 10)

Group 4: Healthy controls (n = 80)

→ no pruritus (NRS in last 24 h = 0), clinically normal skin, no atopic predisposition

Exclusion criteria:

All projects of RU SOMACROSS share common basic exclusion criteria including serious illness requiring immediate intervention, florid psychosis or substance abuse disorder, and acute suicidality.

In addition, the project-specific exclusion criteria are the following:

- Chronic pain syndrome, diagnosis of fibromyalgia, chronic intake of analgesics
- Intake of systemic steroids, systemic immunosuppression, in the past 4 weeks;
- Topical therapy of target area with steroids in the past 2 weeks
- Pregnancy, breast-feeding
- Allergy against substance used for local anaesthesia

Study design: The ideal design to identify chronicity factors for the symptom pruritus would be a prospective longitudinal investigation starting at the first manifestation of the pruritic condition, which is known to persist in a certain percentage of cases (approximately 60-70% of patients; analysed by our own cohort). However, this is not feasible in pruritus for the reasons stated above. We therefore propose a combined design of patients showing acute inflammatory pruritus and a later stage of chronic inflammatory pruritus together mimicking the long-term course of the disease. These groups will be contrasted to patients with chronic pruritus in a non-inflammatory background.

We expect that each group will include patients whose itch symptoms improve. This is considered clinically relevant following a decrease of at least 2 points on the NRS (range 0 – 10).⁵² We also expect that some patients will show less than a 2-point NRS improvement, some will not improve or the symptoms may get worse. We will stratify the patients at 12 months into a PSS group (0-1 points change on the pruritus NRS) and a non-PSS group (decrease of ≥ 2 pruritus NRS points).

This design enables us to identify the similarities and differences between patient groups with acute pruritus and CP and between those with and without PSS:

- a) a cross-sectional comparison between acute AD, chronic AD, CPNL and healthy controls;
- b) a longitudinal investigation of predictive outcome factors in AD and CPNL under treatment according to existing guidelines.

Patients will be followed up for 12 months with three visits (baseline, 6 months, and 12 months). During each visit, we will obtain patient-reported outcomes (RU SOMACROSS core set of questionnaires, pruritus characteristics and course, quality of life, itch questionnaires, scratch pleasure rating questionnaires). Structured clinical interviews, a clinical examination, QST and psychophysical/allodynia testing, biopsies, blood collection and, in a subset of 10 patients from each group, non-invasive MPT-FLIM, hyperspectral imaging (HSI), AFM and TEWL will be performed at each visit (summarized in **Figure 4** and **Table 1**).

- Table 1 approx. here –

-Figure 4 approx here-

Data collection and analyses:

Quantitative sensory testing (QST) allodynia.

Sensitization profiles of patients and healthy volunteers will be analysed using a comprehensive battery for QST and tests for mechanically induced pruritus (allodynia). QST is a validated psychophysical test protocol established in both centres. We will determine thermal and mechanical detection and pain thresholds as well as responses to supra-threshold stimuli. QST reports the gain or loss of function among peripheral nerve fibre populations and may indicate the presence of central sensitization. In allodynia testing, stimuli that usually do not induce itching (e.g., heat or touch) will be tested for their ability to evoke abnormal itching in sensitized participants.

Biopsies, blood

After quantitative sensory testing and allodynia testing, 4-mm biopsies will be obtained from each participant. In CPNL patients, biopsies will be obtained from non-lesional pruritic skin whereas in acute and chronic AD patients, biopsies will be taken from lesional pruritic skin. Biopsies from non-lesional non-pruritic skin will be obtained in all patients and in the healthy controls. An additional biopsy will be obtained from healed non-pruritic skin from matched locations at month 12 from the patients. Biopsies will be divided in two, one part for RNA/protein expression analysis and the other for histopathology.

The fixed biopsy samples will be used to analyse the epidermal and dermal nerve fibre density and anatomy by staining 30 µm sections for the pan-axonal marker PGP9.5. The basement membrane will be visualized by staining for collagen IV in order to determine the precise point at which nerve fibres invade the epidermis.

The unfixed biopsy samples will be used for the detection of relevant pruritogens and mediators (NGF, artemin, semaphorin 3A, IL1β, IL6 and IL31) at the RNA and protein levels. Samples stored in RNA later will be processed by company instructions.

Finally, blood samples from each participant at baseline and from the CP patients at month 12 will be used for the analysis of serum levels of CRP, IL1β, IL6, IL31 and tumour necrosis factor α.

Characterization of CP-related alterations of keratinocyte metabolism and skin barrier integrity

A clinical panel will be enrolled at the study centre UKE for the objective characterization of CP by hyperspectral imaging (HSI) and multiphoton tomography equipped with a fluorescence lifetime imaging module (MPT-FLIM). The panel will comprise 12 patients each with acute AD, chronic AD, and CPNL, and 24 healthy controls. Intravital images will be taken from two lesional or non-lesional skin areas per subject at the volar forearm or antecubital fossa and aligned with photographic documentation and clinical scores gathered by dermatologic consultants. We will evaluate the following outcome parameters: high-resolution images of the epidermal and dermal architecture (e.g., showing the thickness of epidermal layers, cell size, and degree of spongiosis), peripheral blood perfusion and oxygenation/haemoglobin saturation, metabolic status of epidermal skin layers at the single-cell level based on the free-to-bound NADH ratio, and mitochondrial distribution analysis.²⁸

In parallel to HSI and MPT-FLIM measurements, we will investigate skin areas by atomic force microscopy (AFM)²⁹ following the acquisition of stratum corneum samples by tape stripping. Subsequent image analysis will reveal the size of individual corneocytes, intercellular gaps and the number of corneodesmosomes. By combining these data with immunofluorescence

microscopy, we will also identify the tight junction protein claudin-1. To supplement the AFM measurements, we will therefore determine TEWL (CE-certified medical device strictly used in its intended purpose/use) values as a common correlate of skin dryness, disease severity and itchiness.

Assessment and study outcomes

Primary outcome:

Given that the severity of somatic symptoms must be specifically assessed for each symptom in RU SOMACROSS, and that generic instruments are needed for comparisons and common evaluations across projects, somatic symptoms will be measured in two ways:

- a) symptom-specific primary outcome: intensity of pruritus, as assessed by worst NRS score in the past 24 hours
- b) generic instruments: generic assessment of overall symptom severity using the well-established patient health questionnaire 15 (PHQ-15)³⁰ and the NRS for symptom intensity recommended by the EURONET-SOMA group³¹.

Secondary outcomes:

A) Pruritus-specific:

- Pruritus characteristics (intensity, quality, and duration) and course (Itch Controlled Days)
- Pruritus-related quality of life assessed by ItchyQoL
- Scratch pleasure rating (NRS)
- Function of group C and group A nerve fibres (QST, psychophysical results)
- Cutaneous nerve fibre anatomy, biomarker (PCR, immunohistochemistry, blood markers)
- Nanostructure of the skin barrier, metabolic changes in keratinocytes

B) Generic secondary outcome measures:

- interference by additional symptoms, disability and quality of life
- Comorbidity of somatic symptom disorder

Predictors:

- **A) Pruritus-specific:** Pruritus characteristics (intensity, quality, and duration) and course (Itch Controlled Days); pruritus-related cognitions (itching cognition questionnaire);
- **B) Generic predictors:** The set of core instruments reflects the most relevant variables for the development of persistent symptoms, as proposed by the RU's working model.

In addition to the project-specific outcome variables (**Table 1**), we will apply all additional joint core instruments of the RU SOMACROSS (as presented by Löwe et al⁶ in this journal) in our project to contribute to the main research aim of RU SOMACROSS, i.e. to identify risk factors and mechanisms for the persistence of somatic symptoms across diseases.

- Table 1 approx. here-

Measurement points:

- a) Validated itch questionnaires (including pruritus-specific quality of life) and the core instruments of the research unit will be presented to all participants at baseline, 6 months, and 12 months.
- b) Participants will be tested for peripheral and central neuronal sensitization by QST, allodynia testing, and the detection of laboratory inflammation markers (baseline, 6 months, and 12 months). Sensitization patterns will be correlated with findings from (a).

Sample size estimation: Previous data are not available for our outcome measures, so we will conduct an exploratory study. However, we based the sample size on our previous work. Given our previous results, we anticipate normally distributed data for intraepidermal nerve fiber (IENF) density in CP patients with AD (A) and healthy controls (A*)¹¹. Conservative corrected values predicting the reliability of $\mu A = 10$ and $\mu A^* = 14.5$ ($\sigma A = 5$, $\sigma A^* = 6$) lead to a difference ($\mu A - \mu A^*$) of -4.5 and the conservative corrected standard deviation $\sigma = (\sigma A^2 + \sigma A^{*2})^{1/2} = 7.8$. Taking this into account, we propose to reject the hypothesis H_0 ('There is no difference between the groups in IENF; two-sided testing, significance level = 5%) with a power of ~84% and a sample size of 30 per group. We assume similar differences for other comparisons of cutaneous nerve fibre characteristics between the groups, but a valid power calculation is not possible due to the lack of data. These analyses will therefore be interpreted as exploratory (hypothesis generating) rather than confirmatory. To avoid loss of power due to overoptimistic assumptions, we will analyse larger samples ($n = 40$ per group) in the proposed study. For single-cell MPT-FLIM and AFM experiments, we expect (based on preliminary data) a minimal effect size of $f = 1.4$ for the comparison of lesional AD and healthy skin, which leads to nine patients per group (power: $1 - \beta = 0.8$, $\alpha = 0.05$).

Statistical methods: During the first funding phase, we will test paths and associations between psychosocial and biological predisposing and triggering factors with the primary and secondary outcome measures (pruritus-specific as well as generic) using an exploratory approach. Pruritus data are scarce, as is the case for most other diseases investigated by our research group. We therefore included a large number of variables in the first funding phase, enabling us to generate hypotheses for rigorous testing during the second funding phase. The statistical evaluation will be carried out by the biometric experts of the RU SOMACROSS (Z-Project) using cross-sectional (comparisons between the three patient groups and healthy controls) and longitudinal (comparisons across the measurement points) as well as combined approaches, as appropriate. Missing data will be imputed if more than 5% of the data are missing. In accordance to White, Royston, Wood,³² the number of imputations will be chosen dependent on the proportion of missing data. To evaluate subgroup effects, interaction tests will be performed. The statistical analysis of data generated during the first funding phase will reduce the number of predictors and confounders of symptom persistence by removing irrelevant pathways, which will make confirmatory analysis possible during the second funding phase. In the second funding phase, the predictors and confounders identified in the first funding phase will be examined using confirmatory designs. Given that the first funding phase is exploratory, we have not adjusted our statistical methods for multiple testing in order to avoid a loss of power.

Patient and public involvement: It was not appropriate to involve patients or the public in the design of our study. However, the main findings will be published in peer-reviewed journals and made publicly available. In addition, we will communicate scientific results in lay language via press releases, social media, and patient forums.

Impact and contribution to overall project objectives
In this first exploratory phase of the project, we expect to identify biomedical and psychosocial factors contributing to the persistence of pruritus, thus generating a predictive model that we intend to replicate and validate for other skin diseases during a planned second funding phase. There are mechanistic and clinical similarities between fatigue, pain and pruritus. Pruritus is

also well known in kidney and liver diseases. These are conditions which are examined in other projects of the RU SOMACROSS.

In matching our results with those obtained from **all the other projects**, we expect to identify generic mechanisms contributing to the persistence of pruritus, fatigue, pain and other symptoms. Thus, we will contribute to the overall aims of the research group, i.e. to differentiate between disease-specific and generic factors affecting the persistence of symptoms and to generate an overarching model.

ETHICS AND DISSEMINATION

Ethical approval: The study was approved by the Ethics Committees of the Medical Associations Hamburg and Westphalia-Lippe / Westphalian Wilhelms University, Münster, Germany. The study will be conducted in accordance with the WMA Declaration of Helsinki, guidelines for Good Clinical Practice, national and local laws.

The main findings will be published in peer-reviewed journals and made publicly available. In addition, we will communicate scientific results in lay language via press releases, social media, and patient forums.

Furthermore, the ethical considerations published by Löwe et al.⁶ also apply for this project.

AUTHORS' CONTRIBUTIONS

Gudrun Schneider, Stefan W. Schneider, Sonja Ständer conceptualized the study, wrote the project protocol and applied for funding. At present they are supervising the project staff. Stefan Kahnert, Manuel Pereira, Gina Frank, Christian Mess, Volker Huck, Konstantin Agelopoulos are involved in the preparation of the study start and in data collection. All authors contributed to the manuscript and critically revised the manuscript draft.

COMPETING INTERESTS STATEMENT:

Gudrun Schneider, Sonja Ständer, Stefan Kahnert, Manuel P. Pereira, Christian Mess, Volker Huck, Konstantin Agelopoulos, Gina Frank, Stefan W. Schneider have no competing interests to declare.

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Table 1: Pruritus-specific predictors and outcome measures

Predisposing, triggering and maintaining /aggravating factors	Single constructs	Instrument	Months		
			0	6	12
Biological factors	Cellular metabolism	Multiphoton tomographic fluorescence lifetime imaging (MPT-FLIM)	X	X	X
		Hyperspectral imaging (HSI)	X	X	X
	Skin barrier nanostructure	Atomic force microscopy (AFM)	X	X	X
		Trans-epidermal water loss measurement (TEWL)	X	X	X
	Cutaneous nerve fibre anatomy	Biopsies, immunohistochemistry, blood samples	X		X
	Molecular markers	qPCR (NGF, semaphorin 3A, artemin)	X		X
	Indicators of central sensitization	Quantitative sensory testing (QST)	X	X	X
		Allodynia	X	X	X
	Pro-inflammatory marker	Blood: ELISA for relevant marker IL31	X		X
Pruritus-specific outcome variables (assessed via self-report)					
PRIMARY	Pruritus intensity	NRS: worst pruritus in the last 24 hours	X	X	X
SECONDARY	Pruritus-related impairment and life quality	Pruritus intensity scales ItchyQoL, itch controlled days, scratch pleasure rating (NRS)	X	X	X

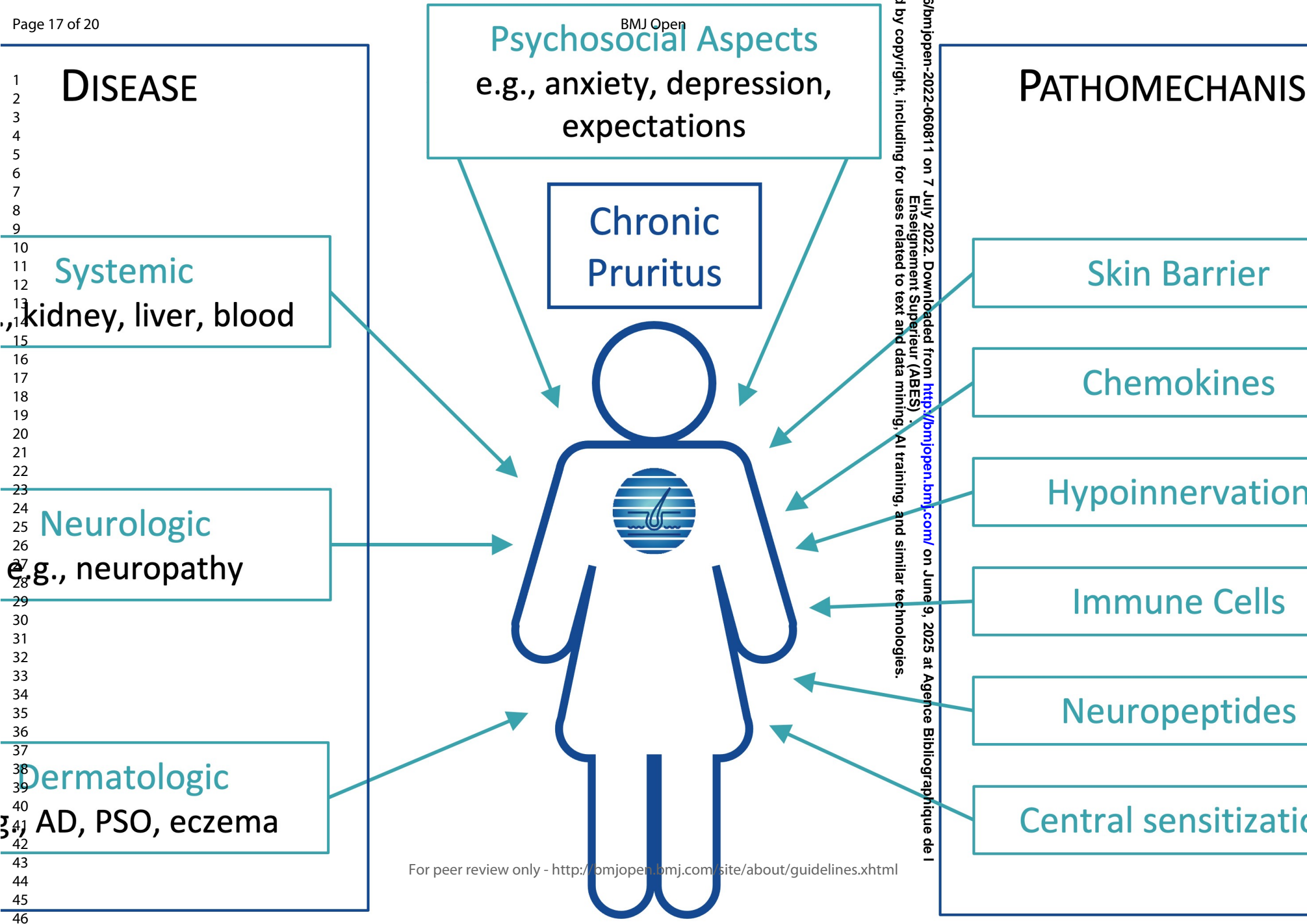
Figure captions:

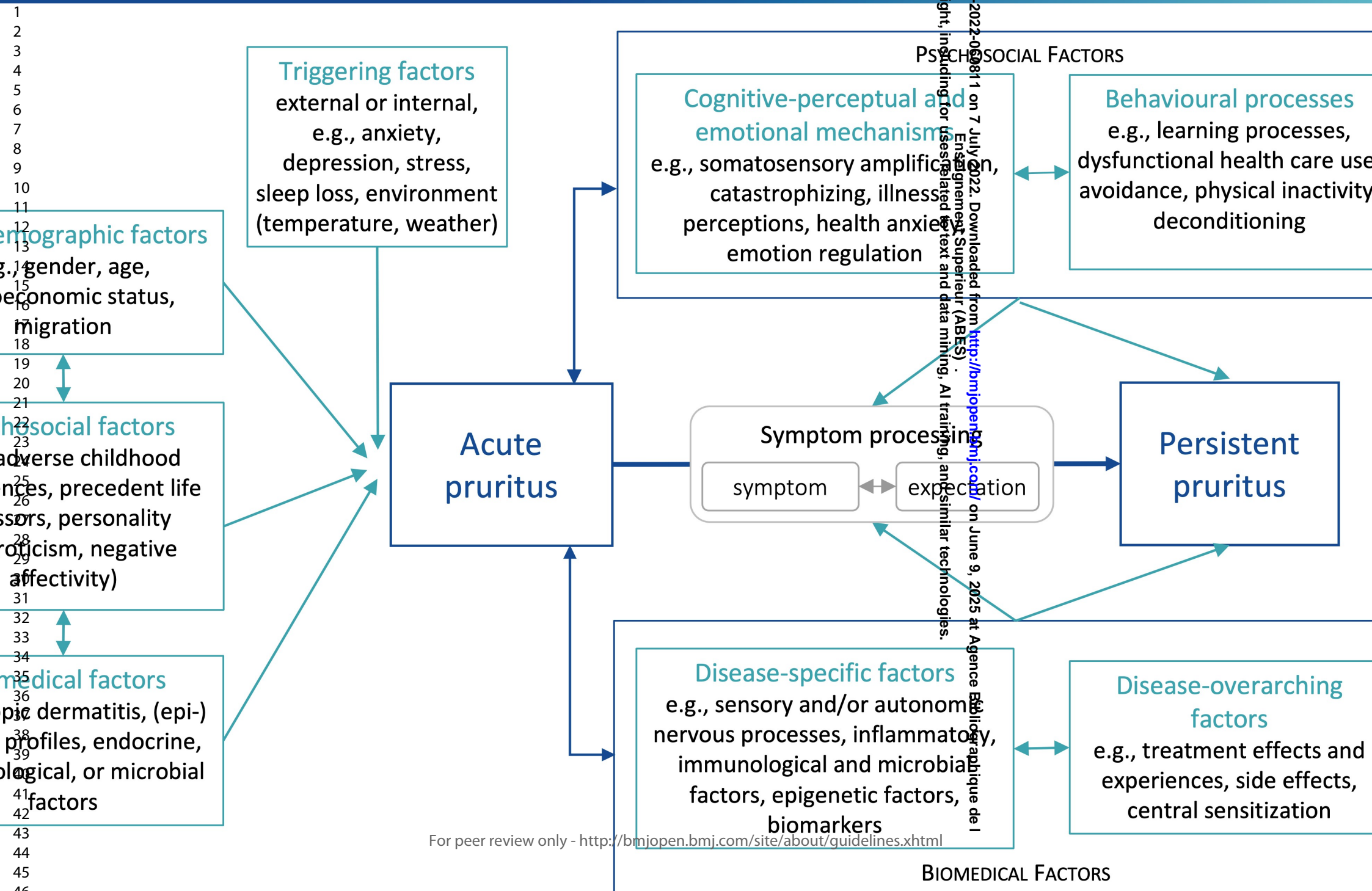
Figure 1: Aetiology and pathomechanisms of chronic pruritus as persistent somatic symptom (PSS)

Figure 2: Working model for pruritus persistence: Risk factors and mechanisms for pruritus persistence as investigated by this project

Figure 3: Study design

Figure 4: An overview of methods and questionnaires applied to correlate psychosocial factors, structural and pathophysiological dysfunctions as risk factors for chronic itching. Schematic skin model: SC = stratum corneum (main skin barrier); SG = stratum granulosum, SS = stratum spinosum; SB = stratum basale; BM = basement membrane; BV = blood vessels; CNF = cutaneous nerve fibres. Psychosocial predisposing, triggering and maintaining/aggravating factors, as well as some of the primary and secondary outcome measures, will be recorded using a comprehensive set of validated and reliable questionnaires and rating scales, which will be applied at each visit. These comprise the core set of questionnaires of the research unit, as well as pruritus-specific questionnaires. Pruritus-specific and generic predictors and outcome measures, including the questionnaires are summarized in Table 1. By applying non-invasive multiphoton tomography equipped with a fluorescence lifetime imaging module (MPT-FLIM), high-resolution morphological imaging of distinct layers of the skin (*morph*) will be combined with fluorescence lifetime imaging-based single-cell metabolic characterization (*FLIM*). Furthermore, hyperspectral imaging (HSI) of the target skin area will facilitate the quantification of oxygenation levels (*StO2 [%]*), haemoglobin saturation of the tissue (*THI*) and peripheral blood perfusion (*NIR*). After tape stripping, atomic force microscopy (AFM) will allow the nanoscale quantification of individual corneocytes, intercellular gaps, corneodesmosomes and claudin-1 levels. PGP9.5 staining followed by fluorescence microscopy will allow the analysis and quantification of intra-epidermal nerve fibres (IENFs). Cutaneous neuroanatomy differs between CP patients and healthy controls but also between different CP entities (upper panel = atopic dermatitis, lower panel = prurigo nodularis). MPT-FLIM, HSI, AFM and IENF will be combined for the analysis of CP-related metabolic changes, disruption of skin barrier integrity and innervation of the skin as novel objective parameters representing the severity of CP.





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Individuals with CP (n = 120) Healthy Controls (n = 80)

Pruritus

1. – 4.

Pruritus

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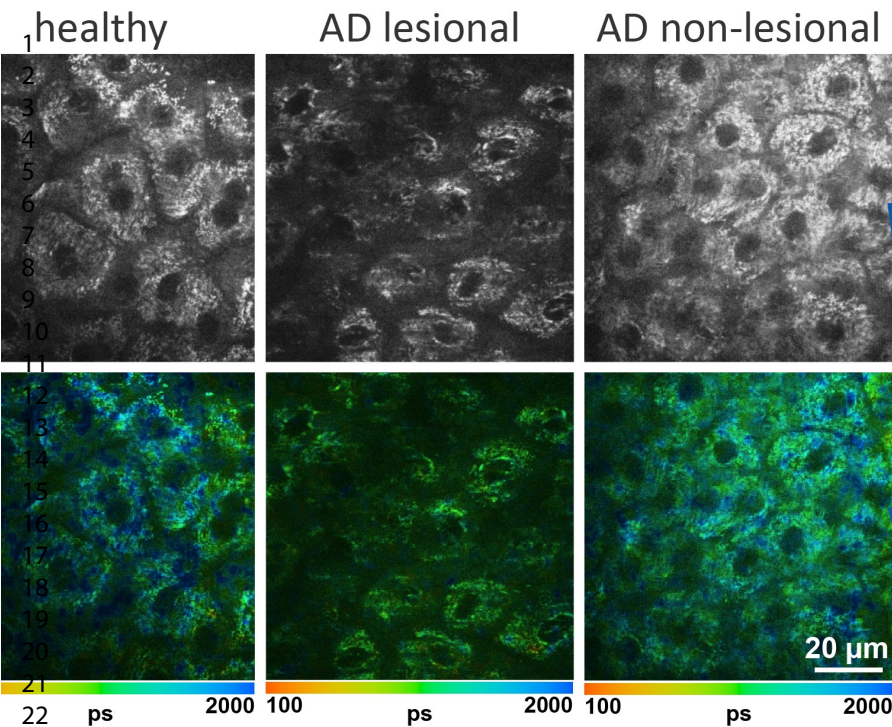
Baseline

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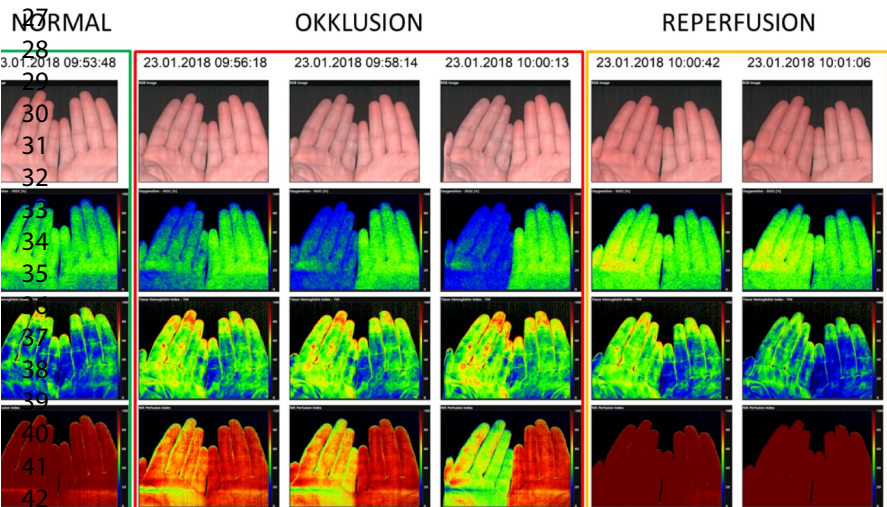
12 months

- Zoom In
1. Psychosocial aspects (Questionnaire)
 2. Neurological analysis (QST, Histology)
 3. Skin barrier and metabolism (MPT-FLIM, AFM)
 4. Pruritogens (blood analysis)

MPT-FLIM

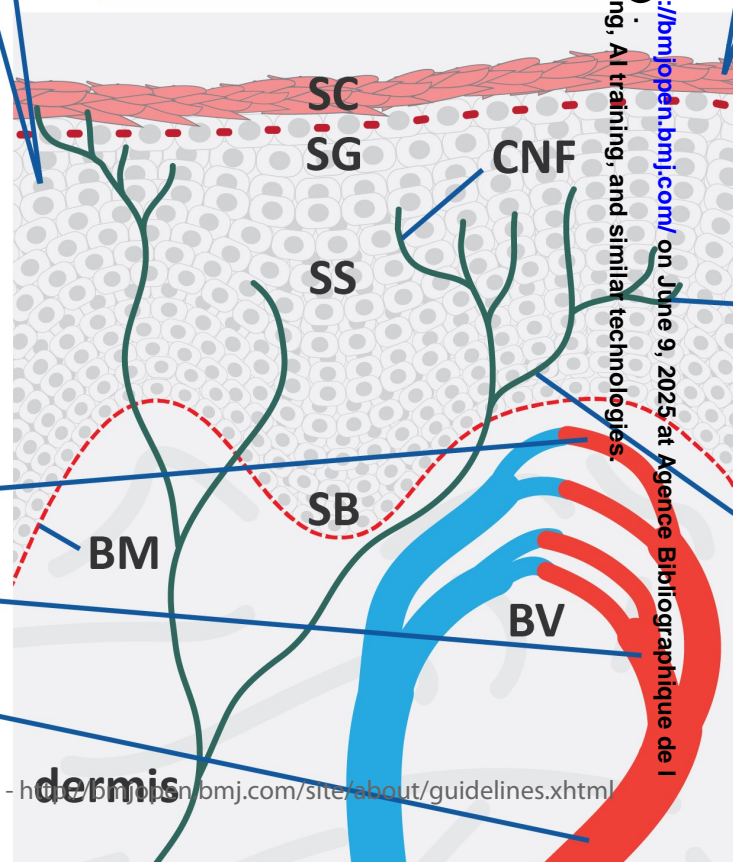


HSI



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Psychosocial mechanisms and risk factors (questionnaire)

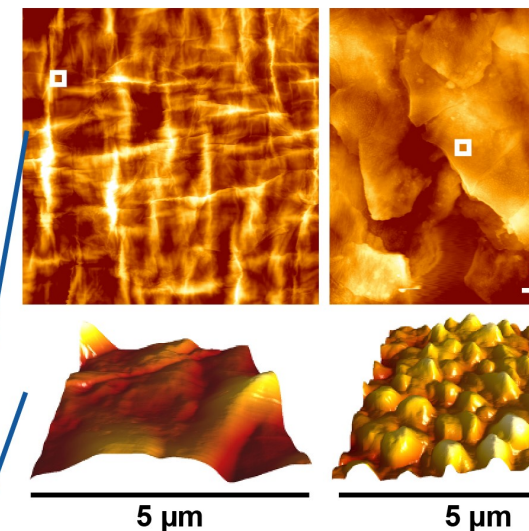


AFM

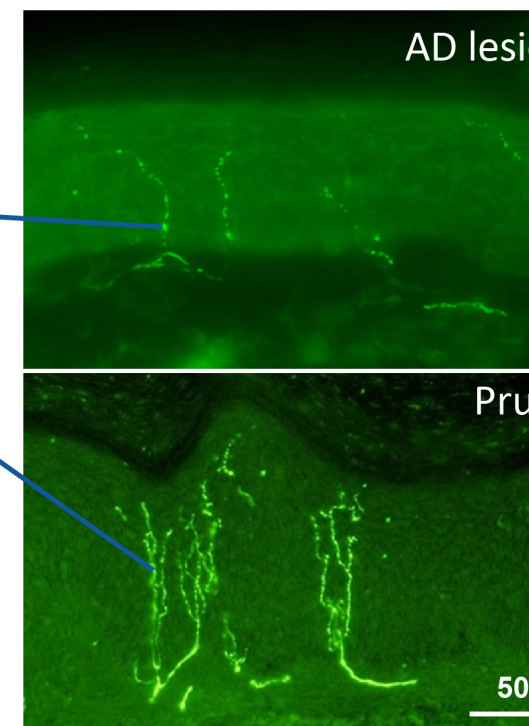
Page 20 of 20

healthy

AD lesion



IENF



BMJ Open

Biological and psychosocial factors associated with the persistence of pruritus symptoms: protocol for a prospective, exploratory observational study in Germany (individual project of the Interdisciplinary SOMACROSS Research Unit [RU 5211])

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Biological and psychosocial factors associated with the persistence of pruritus symptoms: protocol for a prospective, exploratory observational study in Germany (individual project of the Interdisciplinary SOMACROSS Research Unit [RU 5211])

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ABSTRACT

Introduction: Chronic pruritus (CP) is a symptom of dermatologic, neurologic, systemic and psychosomatic diseases. CP has a prevalence of ~20% in the general population and is therefore a significant burden on society, but the transition from acute pruritus to CP is not well understood. It probably involves interactions between biological and psychosocial factors and pruritus-specific risk factors as well as mechanisms shared with other persistent somatic symptoms addressed in other projects of the SOMACROSS Research Unit (RU). Here we aim to identify psychosocial and biological factors and their interactions which might be associated with the persistence of CP with and without immunologic / inflammatory origin, i.e. atopic dermatitis and pruritus on non-inflamed skin. We expect that psychosocial factors relevant to the persistence of symptoms such as fatigue and pain may also show associations to CP.

Methods and analysis: In this prospective, exploratory observational study situated in Germany, three cohorts of 40 patients each with acute exacerbation of atopic dermatitis and chronic atopic dermatitis and 40 CP patients with unaffected skin will be recruited for a comprehensive translational investigation including pruritus-specific and the shared psychosocial assessments of the RU SOMACROSS. Pruritus-specific measures will include questionnaires, quantitative sensory testing, cutaneous nerve fibre morphology, skin barrier morphology, epidermal metabolism, and pruritogen blood levels. Within one year, patients and 80 age- and sex-matched healthy controls will be examined at three time points, allowing cross-sectional comparison and a longitudinal investigation of predictive outcome factors in patients under treatment according to existing guidelines.

Ethics and dissemination: The study has been approved by the ethics committees of Hamburg (2020-10200-BO-ff) and Münster (2020-676-f-S), Germany. All participants are required to provide written informed consent. Findings will be disseminated through peer-reviewed publications, scientific conferences, and involvement of relevant stakeholders, patients and the lay public.

Study registration: German Register for Clinical Studies, DRKS00026646.

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Keywords: Pruritus; Itch; Persistent Somatic Symptoms; Mechanisms; Risk Factors; Expectations; Research Unit; Biopsychosocial Models; Prediction Models

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- A strength of this study is the comprehensive methodological approach (including biological and psychosocial aspects) in three distinct groups of pruritus patients (acute and chronic atopic dermatitis and pruritus on non-lesional skin), which has not been attempted before.
- The novel techniques available in this project (Atomic Force Microscopy [AFM], Hyperspectral Imaging [HIS] and Multiphoton Tomography equipped with a Fluorescence Lifetime Imaging module [MPT-FLIM]), which have not been applied to patients with atopic dermatitis and chronic pruritus on non-lesional skin before, are another strength of the project.
- A further strength is the follow-up over 12 months and the comparison with a control group of healthy participants.
- The methodological crosslink with the other projects of the RU SOMACROSS and the use of a common set of instruments in all projects is a methodological strength, as it is a novel strategy that allows us to compare results between projects and to distinguish between symptom-specific and generic mechanisms.
- A limitation is the fact that, despite investigating multiple potential risk factors and mechanisms of the persistence of chronic pruritus, other variables, which are not addressed in this project, might be relevant.

INTRODUCTION

Chronic pruritus (CP), also known as chronic itch, is defined as the presence of itching that lasts for 6 weeks or more. CP is a symptom in many dermatologic, systemic, neurologic and psychosomatic diseases. It has a high burden on society, with a prevalence of ~20% in the general population and across various medical specialities.^{1,2} The onset and course of CP can be influenced by different psychosomatic aspects and psychologic comorbidities.^{3,4} CP therefore is classified as a persistent somatic symptom (PSS) and fits conceptual models of PSS aetiology.⁵ Like other forms of PSS, CP is thought to arise from complex interactions between the brain, body and environment (**Figure 1**). Thus, this fits well into the interdisciplinary Research Unit SOMACROSS, which has been presented by Löwe et al. ⁶ in this journal.

- Figure 1 approx. here-

Not all of the biological, psychological and social factors and their interactions that are associated with the transition from acute pruritus to CP are well understood. Persistent CP is likely to be associated with pruritus-specific pathophysiological, psychological and social factors and also with mechanisms that are shared with other forms of PSS. A comprehensive model of the aetiology and persistence of CP still needs to be validated.

-Figure 2 approx.here-

Acute pruritus may be triggered by chemical, physical or mechanical stimuli. Pruritus may be induced by pruritogens released from inflammatory cells and keratinocytes, as well as nerve fibres of the peripheral or central nervous system. Cutaneous group C and group A nerve fibres carry corresponding receptors for interleukins (ILs) such as IL31 and IL4, histamine, or neuropeptides such as substance P and somatostatin.^{1,7} Atopic dermatitis (AD) is the most prevalent pruritic dermatologic disease ⁸ and the most widely studied model of immunologic and skin-barrier related pruritus mechanisms. AD can occur in acute phases, and chronic phases with persistent pruritus and subacute eczema. At all stages, patients suffer from intense pruritus regardless of the severity of the eczema.⁹ On the other hand, CP may also occur on non-inflamed, non-lesional skin (CPNL), e.g. due to systemic, neurologic or other diseases. Skin barrier dysfunction is widely considered to be the primary event in AD and is pivotal for pruritus.¹⁰ The outermost layer of the epidermis (stratum corneum) is the most relevant skin layer for the cutaneous barrier function. In AD skin lesions, the expression of tight junction proteins such as claudin-1 is reduced, and the stratum corneum becomes 'leaky' and shows dramatic morphological and structural changes shown by atomic force microscopy (AFM). Furthermore, the skin barrier is also impaired in clinically non-lesional AD skin. The skin barrier dysfunction, along with a biphasic Th2/Th1 response, the release of pruritogenic cytokines, epidermal hypoinnervation, and neuronal hypersensitivity, are considered essential for the induction and most likely also the persistence of pruritus, while the associations with psychosocial factors are unclear.¹¹

Neural hypersensitivity of the peripheral and/or central nervous system has been demonstrated in AD and CP of other origin.^{1,4,12} In this state, nerve fibres are more vulnerable to pruritogens, the corresponding receptors / ion channels have a lower activation threshold, and neuromediator production in nerve fibres is enhanced⁴. E.g., cutaneously applied pruritogens evoke pruritus that is more intense and longer lasting than usual (hyperknesis).^{1,13} In contrast, alloknesis describes the induction of pruritus by non-pruritogenic stimuli such as mechanical triggers.

Mechanisms associated with neuronal hypersensitivity are largely unknown but may include chronic stimulation with inflammatory pruritogens such as IL31 and IL4,⁴ permanent scratching,^{14,15} the depletion of cutaneous Merkel cells,¹⁶ psychological and/or social factors.¹⁷ Furthermore, several previous studies have shown that social and psychological factors are related to an enhanced perception of pruritus (**Figure 2**).

Positive and negative expectations, which can also be induced by corresponding placebo and nocebo instructions, have been shown to influence the intensity of pruritus, scratching behaviour and even the physiologic skin reactions after experimentally induced pruritus in healthy controls^{18,19} and in patients with AD²⁰. Anxiety, depression²¹ and personality traits such as neuroticism (i.e., emotional instability with the tendency to experience more negative emotions), low agreeableness^{21,22} and low self-efficacy²³ were associated with a stronger perception of pruritus in patients with AD, psoriasis, and in healthy controls. Self-reported pruritus has been repeatedly linked to self-reported perceptions of stress and stressful life events in population samples^{20, 23} and also in AD patients.²⁴⁻²⁷

Biologically, patients with AD may show a response to stress in terms of T-cell and mast cell activation, expression and release of neurogenic inflammatory mediators, and disruption of normal epidermal barrier function.²⁷ Stress has been shown to deteriorate the skin barrier by activating 11beta-Hydroxysteroid Dehydrogenase 1 and the HPA axis.²⁸ The association between stress and pruritus can also be mediated by negative pruritus-associated cognitions and expectations in AD patients (**Figure 2**).²⁶

Taken together, the results of multiple studies confirm psychological factors to be risk factors for an enhanced perception of pruritus, but the extent to which these factors are associated with the persistence and chronicity of CP remains unclear (**Figure 1**).

Novelty of the study

CP has biological, psychological and social dimensions and the interactions between these factors are poorly understood. Data gathered so far were mostly derived from cross-sectional studies not set out to identify such interactions. We will therefore apply a comprehensive set of instruments to analyse biomarkers of the skin and blood, the function and anatomical structure of the skin barrier, the peripheral nervous system and its interaction with the central nervous system, as well as psychological and social factors in two key forms of CP (inflammatory and non-inflammatory). In addition, we will follow the course of pruritus over 12 months and relate the progression of the disease to the above parameters in order to identify psychosocial and biological factors that are associated with the persistence of symptoms. Such a comprehensive approach has not been attempted before, and we will increase the value of the work by studying three distinct groups (two inflammatory and one non-inflammatory) of pruritus patients. The crosslink with the other projects of the RU SOMACROSS and the use of one common set of instruments in all projects is a completely new strategy that allows us to

compare results between projects and to distinguish between symptom-specific and generic mechanisms. The novel techniques available in this project have not been applied to patients with AD or CPNL before.

Objectives

The overall aim of the project is to identify psychosocial and biological factors (and their interactions) that are associated with the persistence of symptoms in CP. The psychosocial factors relevant to the persistence of symptoms in conditions such as fatigue and pain may also be associated with CP. Therefore, the project has the following main objectives:

1. To characterize psychosocial factors: (i) associated with the persistence of CP in general, and (ii) the persistence of CP of cutaneous inflammatory and non-inflammatory origin in particular.
2. To determine whether there are associations between psychosocial risk factors and specific peripheral and/or central neuronal sensitization patterns in CP.
3. To determine co-variation of modifiable psychosocial risk factors and biological factors (e.g., cellular metabolism and skin barrier function).

Hypothesis

Psychosocial (personality, stress, expectations, catastrophizing, illness perceptions, anxiety, depression) and biological factors (central sensitization processes, cutaneous neuroanatomy, cellular metabolism, skin barrier function) interact in promoting the persistence of pruritus.

METHODS AND ANALYSIS

Study design and participants

Participants

Patients with AD will be included as a model of cutaneous inflammatory pruritus, whereas patients with CPNL (CP with pruritus on non-lesional skin) will be included as a model of non-inflammatory pruritus. AD starts in childhood and we cannot capture the long-term follow up starting at the onset of the disease, so we will recruit two stages of AD: a group of adult patients (group 1; n = 40) presenting with acute AD (after a manifestation-free interval of at least 6 months), and a group of adult patients (group 2; n = 40) presenting with chronic AD. These groups will be subject to cross-sectional and longitudinal comparisons. We will also recruit CPNL patients (group 3; n = 40) in which CP has typically developed over the course of several years. We will compare the patient groups cross-sectionally with age/sex-matched skin-healthy controls (group 4; n = 80) (Figure 3).

- Figure 3 approx.here -

Inclusion criteria

All projects of RU SOMACROSS share common basic inclusion criteria, i.e.: age ≥18 years, sufficient oral and written German language proficiency to complete self-report questionnaires and interviews and written informed consent. In addition to these common criteria, the project-specific inclusion criteria are listed below.

Group 1: CP in acute AD (n = 40)

→ acute eczema for ≤ 4 weeks at inclusion, no eczema in the previous 6 months

Group 2: CP in chronic AD (n = 40)

→ AD and CP for at least 6 weeks, chronic eczema

Group 3: CP in CPNL (n = 40)

→ CP for at least 6 weeks, clinically no dermatosis, no prurigo

Groups 1–3: pruritus intensity ≥ 5 , worst intensity in last 24 h on numeric rating scale (NRS, range 0 – 10)

Group 4: Healthy controls (n = 80)

→ no pruritus (NRS in last 24 h = 0), clinically normal skin, no atopic predisposition

Exclusion criteria

All projects of RU SOMACROSS share common basic exclusion criteria including serious illness requiring immediate intervention, florid psychosis or substance abuse disorder, and acute suicidality.

In addition, the project-specific exclusion criteria are the following:

- Chronic pain syndrome, diagnosis of fibromyalgia, chronic intake of analgesics
- Intake of systemic steroids, systemic immunosuppression in the past 4 weeks
- Topical therapy of target area with steroids in the past 2 weeks
- Pregnancy, breast-feeding
- Allergy against substance used for local anaesthesia.

We included chronic pain conditions including fibromyalgia, as well as intake of analgesics as exclusion criteria, since these conditions and drugs may affect functional assessments of quantitative sensory testing and allodynia. Additionally, we included certain topical and systemic agents (namely systemic steroids, systemic immunosuppressants and topical steroids) as exclusion criteria, as these drugs may affect the expression of relevant markers/genes in the skin and make difficult the interpretation of findings obtained from skin biopsies. Allergy to local anesthetics is also an exclusion criterion, since local anesthesia is needed in order to perform the skin biopsies

Study design

The ideal design to identify chronicity factors for the symptom pruritus would be a prospective longitudinal investigation starting at the first manifestation of the pruritic condition, which is known to persist in a certain percentage of cases (approximately 60-70% of patients; analysed by our own cohort). However, this is not feasible in pruritus for the reasons stated above. We therefore propose a combined design of patients showing acute inflammatory pruritus and a later stage of chronic inflammatory pruritus together mimicking the long-term course of the disease. These groups will be contrasted to patients with chronic pruritus in a non-inflammatory background and followed up for 1 year. Therefore, the study design is prospective, exploratory and observational.

We expect that each group will include patients whose itch symptoms improve. This is considered clinically relevant following a decrease of at least 2 points on the NRS (range 0 – 10). We also expect that some patients will show less than a 2-point NRS improvement, some will not improve or the symptoms may get worse. We will divide the patients at 12 months into a PSS group (0-1 points change on the pruritus NRS) and a non-PSS group (decrease of ≥ 2 pruritus NRS points).

This design enables us to identify the similarities and differences between patient groups with acute pruritus and CP and between those with and without PSS:

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3 a) a cross-sectional comparison between acute AD, chronic AD, CPNL and healthy controls;

4 b) a longitudinal investigation of predictive outcome factors in AD and CPNL under treatment

5 according to existing guidelines.

6

7 Patients will be followed up for 12 months with three visits (baseline, 6 months, and 12 months).

8 During each visit, we will obtain patient-reported outcomes (RU SOMACROSS core set of

9 questionnaires, pruritus characteristics and course, quality of life, itch questionnaires, scratch

10 pleasure rating questionnaires). Structured clinical interviews, a clinical examination, QST and

11 psychophysical/alloknesis testing, biopsies, blood collection and, in a subset of 10 patients

12 from each group, non-invasive MPT-FLIM, hyperspectral imaging (HSI), AFM and TEWL will

13 be performed at each visit (summarized in **Figure 4** and **Table 1**).

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19 **Table 1: Pruritus-specific predictors and outcome measures**

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Predisposing, triggering and maintaining /aggravating factors	Single constructs	Instrument	Months		
			0	6	12
Biological factors	Cellular metabolism	Multiphoton tomographic fluorescence lifetime imaging (MPT-FLIM)	X	X	X
		Hyperspectral imaging (HSI)	X	X	X
	Skin barrier nanostructure	Atomic force microscopy (AFM)	X	X	X
		Trans-epidermal water loss measurement (TEWL)	X	X	X
	Cutaneous nerve fibre anatomy	Biopsies, immunohistochemistry, blood samples	X		X
	Molecular markers	qPCR (NGF, semaphorin 3A, artemin)	X		X
	Indicators of central sensitization	Quantitative sensory testing (QST)	X	X	X
		Alloknesis	X	X	X
	Pro-inflammatory marker	Blood: ELISA for relevant marker IL31	X		X
Pruritus-specific outcome variables (assessed via self-report)					
PRIMARY	Pruritus intensity	NRS: worst pruritus in the last 24 hours	X	X	X
SECONDARY	Pruritus-related impairment and life quality	Pruritus intensity scales ItchyQoL, itch controlled days, scratch pleasure rating (NRS)	X	X	X

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50 -Figure 4 appox here-

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52 Data collection and analyses

53 Sociodemographic and psychosocial variables

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55 In order to assure comparability of the projects within the Research unit, this study employs

56 the same questionnaires to assess sociodemographic and psychosocial variables as the other

57 projects of the Research Unit Somacross- the so-called “core set of instruments”. These have

58 been listed and described in detail in the publication of Löwe et al.⁶ in this journal. The “core

59 set” of instruments assesses socipodemographic and psychosocial factors, cognitive-

60 perceptual and emotional mechanisms and behavioral factors by well-established and

validated self-assessment questionnaires. For details, please consult Löwe et al.⁶ in this journal.

Quantitative sensory testing (QST) allodynia

Sensitization profiles of patients and healthy volunteers will be analysed using a comprehensive battery for QST and tests for mechanically induced pruritus (allodynia). QST is a validated psychophysical test protocol established in both centres. We will determine thermal and mechanical detection and pain thresholds as well as responses to supra-threshold stimuli. QST reports the gain or loss of function among peripheral nerve fibre populations and may indicate the presence of central sensitization. In allodynia testing, stimuli that usually do not induce itching (e.g., heat or touch) will be tested for their ability to evoke abnormal itching in sensitized participants.

Biopsies, blood

After quantitative sensory testing and allodynia testing, 4-mm biopsies will be obtained from each participant. In CPNL patients, biopsies will be obtained from non-lesional pruritic skin whereas in acute and chronic AD patients, biopsies will be taken from lesional pruritic skin. Biopsies from non-lesional non-pruritic skin will be obtained in all patients and in the healthy controls. An additional biopsy will be obtained from healed non-pruritic skin from matched locations at month 12 from the patients. The biopsies at month 12 will be performed in the same anatomical area to avoid inter patient differences.

Biopsies will be divided in two, one part for RNA/protein expression analysis and the other for histopathology.

The fixed biopsy samples will be used to analyse the epidermal and dermal nerve fibre density and anatomy by staining 30 µm sections for the pan-axonal marker PGP9.5. The basement membrane will be visualized by staining for collagen IV in order to determine the precise point at which nerve fibres invade the epidermis.

The unfixed biopsy samples will be used for the detection of relevant pruritogens and mediators (NGF, artemin, semaphorin 3A, IL1β, IL6 and IL31) at the RNA and protein levels. Samples stored in RNA later will be processed by company instructions.

Finally, blood samples from each participant at baseline and from the CP patients at month 12 will be used for the analysis of serum levels of CRP, IL1β, IL6, IL31 and tumour necrosis factor α.

Characterization of CP-related alterations of keratinocyte metabolism and skin barrier integrity

A clinical panel will be enrolled at the study centre UKE for the objective characterization of CP by hyperspectral imaging (HSI) and multiphoton tomography equipped with a fluorescence lifetime imaging module (MPT-FLIM). The panel will comprise 12 patients each with acute AD, chronic AD, and CPNL, and 24 healthy controls. Intravital images will be taken from two lesional or non-lesional skin areas per subject at the volar forearm or antecubital fossa and aligned with photographic documentation and clinical scores gathered by dermatologic consultants. We will evaluate the following outcome parameters: high-resolution images of the epidermal and dermal architecture (e.g., showing the thickness of epidermal layers, cell size, and degree of spongiosis), peripheral blood perfusion and oxygenation/haemoglobin saturation, metabolic status of epidermal skin layers at the single-cell level based on the free-to-bound NADH ratio, and mitochondrial distribution analysis.²⁹

In parallel to HSI and MPT-FLIM measurements, we will investigate skin areas by atomic force microscopy (AFM)³⁰ following the acquisition of stratum corneum samples by tape stripping.

Subsequent image analysis will reveal the size of individual corneocytes, intercellular gaps and the number of corneodesmosomes. By combining these data with immunofluorescence microscopy, we will also identify the tight junction protein claudin-1. To supplement the AFM measurements, we will therefore determine TEWL (CE-certified medical device strictly used in its intended purpose/use) values as a common correlate of skin dryness, disease severity and itchiness.

Assessment and study outcomes

Primary outcome

Given that the severity of somatic symptoms must be specifically assessed for each symptom in RU SOMACROSS, and that generic instruments are needed for comparisons and common evaluations across projects, somatic symptoms will be measured in two ways:

- a) symptom-specific primary outcome: intensity of pruritus, as assessed by worst NRS score in the past 24 hours
- b) generic instruments: generic assessment of overall symptom severity using the well-established patient health questionnaire 15 (PHQ-15)³¹ and the NRS for symptom intensity recommended by the EURONET-SOMA group³².

Secondary outcomes

A) Pruritus-specific:

- Pruritus characteristics (intensity, quality, and duration) and course (Itch Controlled Days)
- Pruritus-related quality of life assessed by ItchyQoL
- Scratch pleasure rating (NRS)
- Function of group C and group A nerve fibres (QST, psychophysical results)
- Cutaneous nerve fibre anatomy, biomarker (PCR, immunohistochemistry, blood markers)
- Nanostructure of the skin barrier, metabolic changes in keratinocytes

B) Generic secondary outcome measures:

- interference by additional symptoms, disability and quality of life
- Comorbidity of somatic symptom disorder

Predictors

- **A) Pruritus-specific:** Pruritus characteristics (intensity, quality, and duration) and course (Itch Controlled Days);
- **B) Generic predictors:** The set of core instruments reflects the most relevant variables for the development of persistent symptoms, as proposed by the RU's working model.

In addition to the project-specific outcome variables (**Table 1**), we will apply all additional joint core instruments of the RU SOMACROSS (as presented by Löwe et al⁶ in this journal) in our project to contribute to the main research aim of RU SOMACROSS, i.e. to identify risk factors and mechanisms for the persistence of somatic symptoms across diseases.

- Table 1 approx. here-

Measurement points

- a) Validated itch questionnaires (including pruritus-specific quality of life) and the core instruments of the research unit will be presented to all participants at baseline, 6 months, and 12 months.

- b) Participants will be tested for peripheral and central neuronal sensitization by QST, allodynia testing, and the detection of laboratory inflammation markers (baseline, 6 months, and 12 months). Sensitization patterns will be correlated with findings from (a).

Sample size estimation

Previous data are not available for our outcome measures, so we will conduct an exploratory study. However, we based the sample size on our previous work. Given our previous results, we anticipate normally distributed data for intraepidermal nerve fiber (IENF) density in CP patients with AD (A) and healthy controls (A*)¹². Conservative corrected values predicting the reliability of $\mu_A = 10$ and $\mu_{A^*} = 14.5$ ($\sigma_A = 5$, $\sigma_{A^*} = 6$) lead to a difference ($\mu_A - \mu_{A^*}$) of -4.5 and the conservative corrected standard deviation $\sigma = (\sigma_A^2 + \sigma_{A^*}^2)^{1/2} = 7.8$. Taking this into account, we propose to reject the hypothesis H_0 ('There is no difference between the groups in IENF; two-sided testing, significance level = 5%) with a power of ~84% and a sample size of 30 per group. We assume similar differences for other comparisons of cutaneous nerve fibre characteristics between the groups, but a valid power calculation is not possible due to the lack of data. These analyses will therefore be interpreted as exploratory (hypothesis generating) rather than confirmatory. To avoid loss of power due to overoptimistic assumptions, we will analyse larger samples ($n = 40$ per group) in the proposed study. For single-cell MPT-FLIM and AFM experiments, we expect (based on our preliminary data) a minimal effect size of $f = 1.4$ for the comparison of lesional AD and healthy skin³³, which leads to nine patients per group (power: $1 - \beta = 0.8$, $\alpha = 0.05$).

Statistical methods

During the first funding phase, we will test paths and associations between psychosocial and biological predisposing and triggering factors with the primary and secondary outcome measures (pruritus-specific as well as generic) using an exploratory approach. Pruritus data are scarce, as is the case for most other diseases investigated by our research group. We therefore included a large number of variables in the first funding phase, enabling us to generate hypotheses for rigorous testing during the second funding phase. The statistical evaluation will be carried out by the biometric experts of the RU SOMACROSS (Z-Project) using cross-sectional (comparisons between the three patient groups and healthy controls) and longitudinal (comparisons across the measurement points) as well as combined approaches, as appropriate. Missing data will be imputed if more than 5% of the data are missing. In accordance to White, Royston, Wood,³⁴ the number of imputations will be chosen dependent on the proportion of missing data. To evaluate subgroup effects, interaction tests will be performed. The statistical analysis of data generated during the first funding phase will reduce the number of predictors and confounders of symptom persistence by removing irrelevant pathways, which will make confirmatory analysis possible during the second funding phase. In the second funding phase, the predictors and confounders identified in the first funding phase will be examined using confirmatory designs. Given that the first funding phase is exploratory, we have not adjusted our statistical methods for multiple testing in order to avoid a loss of power.

Current status and timeline of the study

Patient recruitment started in October 2021 and the first patients have been included. We expect to complete the study within 4 years. The work schedule and milestones are reported in Figure 5.

-Figure 5 approx. here-

Patient and public involvement

It was not appropriate to involve patients or the public in the design of our study. However, the main findings will be published in peer-reviewed journals and made publicly available. In addition, we will communicate scientific results in lay language via press releases, social media, and patient forums.

Impact and contribution to overall project objectives

In this first exploratory phase of the project, we expect to identify biomedical and psychosocial factors associated with the persistence of pruritus, thus generating a predictive model that we intend to replicate and validate for other skin diseases during a planned second funding phase. There are mechanistic and clinical similarities between fatigue, pain and pruritus. Pruritus is also well known in kidney and liver diseases. These are conditions which are examined in other projects of the RU SOMACROSS. The data generated in the first funding phase are relevant for our objectives and will allow us to generate hypotheses on the mechanisms of pruritus persistence, which will be tested during the second funding phase.

In matching our results with those obtained from all the other projects, we expect to identify generic mechanisms associated with the persistence of pruritus, fatigue, pain and other symptoms. Thus, we will contribute to the overall aims of the research group, i.e. to differentiate between disease-specific and generic factors affecting the persistence of symptoms and to generate an overarching model.

ETHICS AND DISSEMINATION

The study was approved by the Ethics Committees of the Medical Associations Hamburg (2020-10200-BO-ff) and Westphalia-Lippe / Westphalian Wilhelms University, Münster (2020-676-f-S), Germany. The study will be conducted in accordance with the WMA Declaration of Helsinki, guidelines for Good Clinical Practice, national and local laws. All participants are required to provide written informed consent. Furthermore, the ethical considerations published by Löwe et al.⁶ also apply for this project.

The main findings will be published in peer-reviewed journals and made publicly available. In addition, we will communicate scientific results in lay language via press releases, social media, and patient forums.

Contributors

Gudrun Schneider, Stefan W. Schneider, Sonja Ständer conceptualized the study, wrote the project protocol and applied for funding. At present they are supervising the project staff. Stefan Kahnert, Manuel Pereira, Gina Frank, Christian Mess, Volker Huck, Konstantin Agelopoulos are involved in the preparation of the study start and in data collection. All authors contributed to the manuscript and critically revised the manuscript draft.

Competing interests

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Gudrun Schneider, Sonja Ständer, Stefan Kahnert, Manuel P. Pereira, Christian Mess, Volker Huck, Konstantin Agelopoulos, Gina Frank, Stefan W. Schneider have no competing interests to declare.

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Figure captions:

Figure 1: Aetiology and pathomechanisms of chronic pruritus as persistent somatic symptom (PSS)

Figure 2: Working model for pruritus persistence: Risk factors and mechanisms for pruritus persistence as investigated by this project

Figure 3: Study design

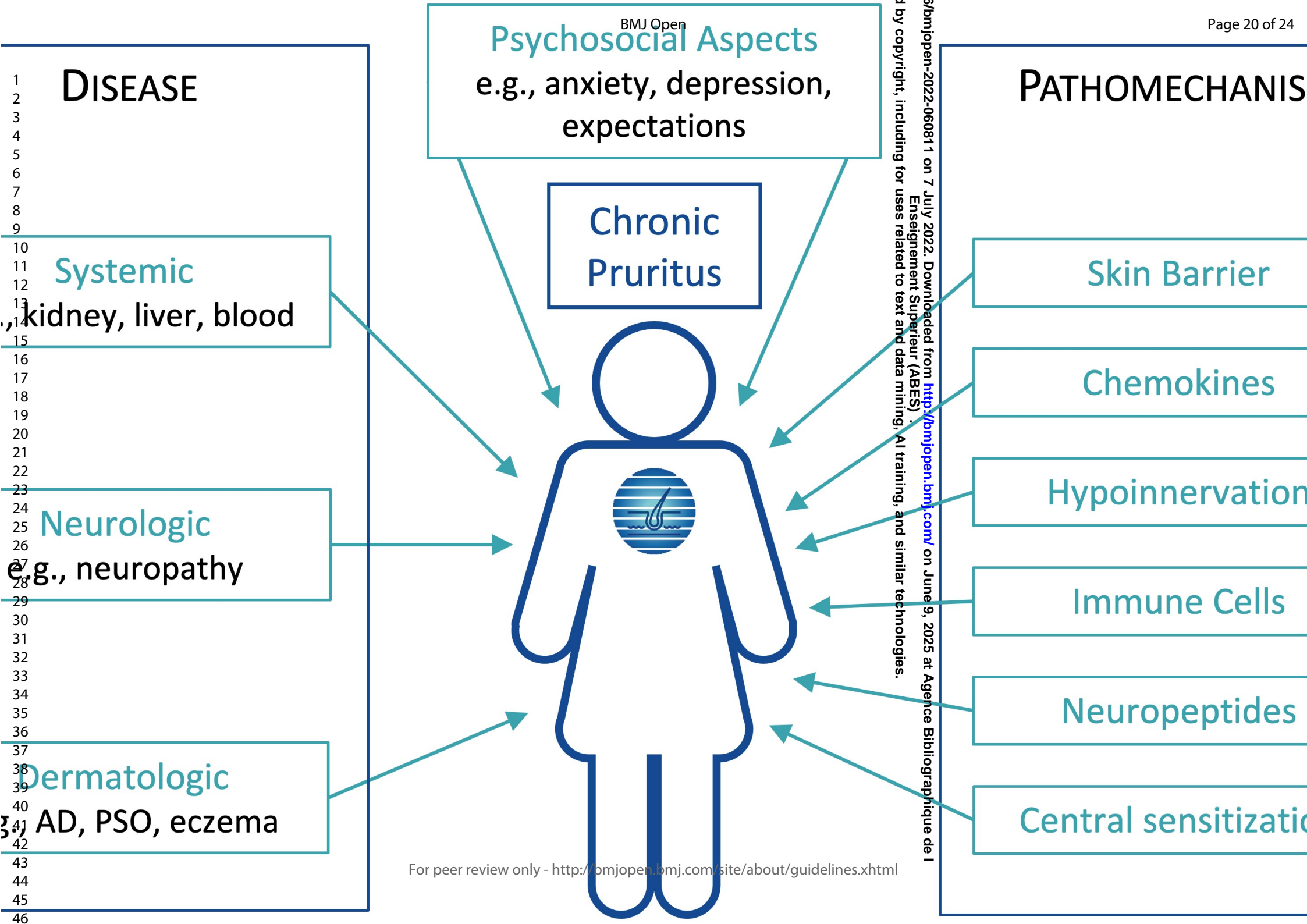
Figure 4: An overview of methods and questionnaires applied to correlate psychosocial factors, structural and pathophysiological dysfunctions as risk factors for chronic itching

Schematic skin model: SC = stratum corneum (main skin barrier); SG = stratum granulosum, SS = stratum spinosum; SB = stratum basale; BM = basement membrane; BV = blood vessels; CNF = cutaneous nerve fibres. Psychosocial predisposing, triggering and maintaining/aggravating factors, as well as some of the primary and secondary outcome measures, will be recorded using a comprehensive set of validated and reliable questionnaires and rating scales, which will be applied at each visit. These comprise the core set of questionnaires of the research unit, as well as pruritus-specific questionnaires. Pruritus-specific and generic predictors and outcome measures, including the questionnaires are summarized in Table 1. By applying non-invasive multiphoton tomography equipped with a fluorescence lifetime imaging module (MPT-FLIM), high-resolution morphological imaging of distinct layers of the skin (*morph*) will be combined with fluorescence lifetime imaging-based single-cell metabolic characterization (*FLIM*). Furthermore, hyperspectral imaging (HSI) of the target skin area will facilitate the quantification of oxygenation levels (*StO2 [%]*), haemoglobin saturation of the tissue (*THI*) and peripheral blood perfusion (*NIR*). After tape stripping, atomic force microscopy (AFM) will allow the nanoscale quantification of individual corneocytes, intercellular gaps, corneodesmosomes and claudin-1 levels. PGP9.5 staining followed by fluorescence microscopy will allow the analysis and quantification of intra-epidermal nerve fibres (IENFs). Cutaneous neuroanatomy differs between CP patients and healthy controls but also between different CP entities (upper panel = atopic dermatitis, lower panel = prurigo nodularis). MPT-FLIM, HSI, AFM and IENF will be combined for the analysis of CP-related metabolic changes, disruption of skin barrier integrity and innervation of the skin as novel objective parameters representing the severity of CP.

Figure 5: Timetable and milestones of the project

- M1. Pruritus/psychosocial questionnaires and clinical data at baseline completed (month 27)
- M2. Last patient, last visit; questionnaires and clinical data assessments completed (month 39)
- M3. First correlations of collected FLIM / IENF data (month 20)
- M4. First correlations of biological and clinical baseline data (month 29)
- M5. Biological data assessment completed (month 46)
- M6. Correlation of questionnaires, clinical and biological data completed (month 46)
- M7. Stratification of patients into PSS/non-PSS and correlation of data at month 12 (month 46)
- M8. Publication (month 48)

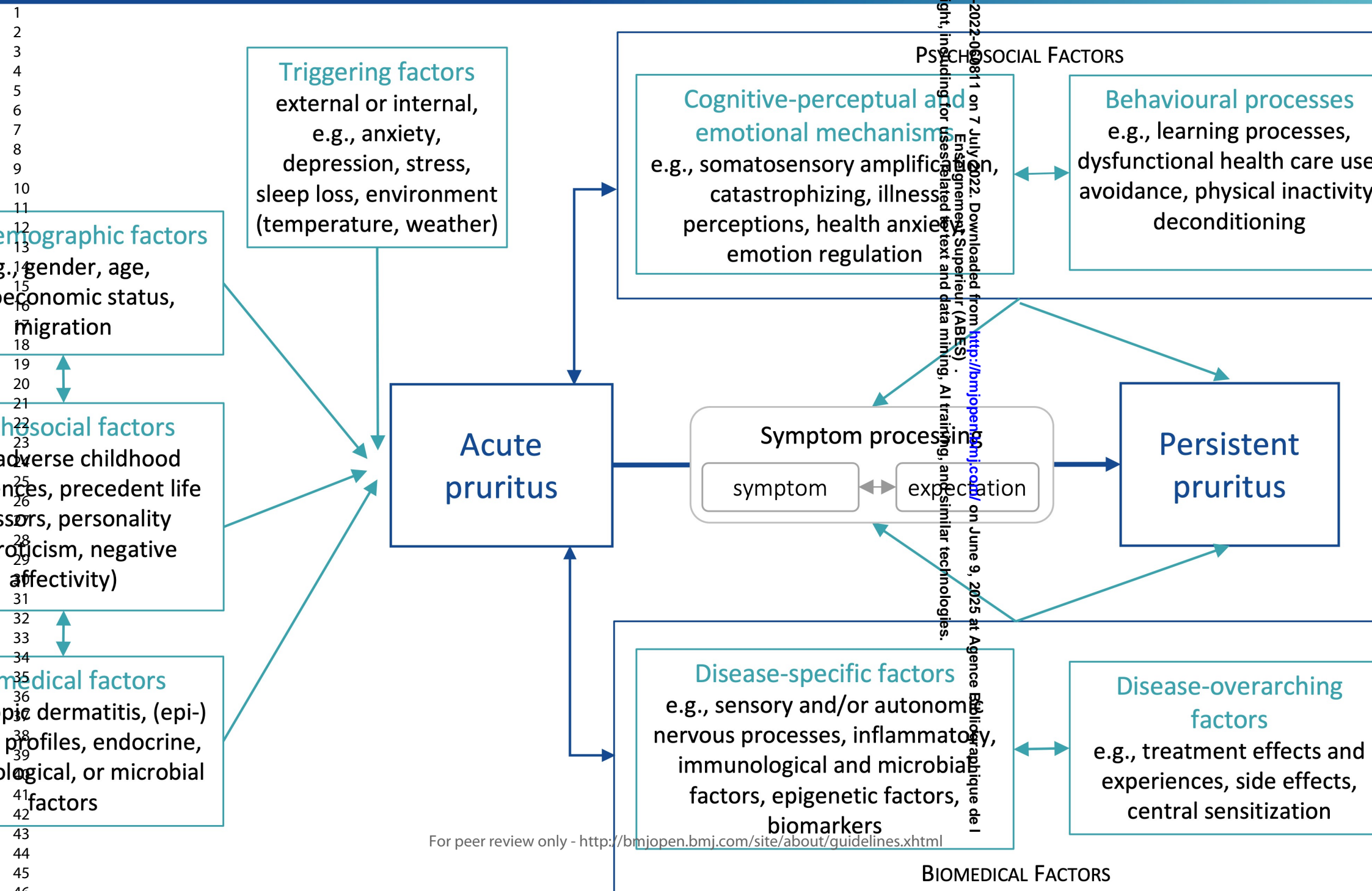
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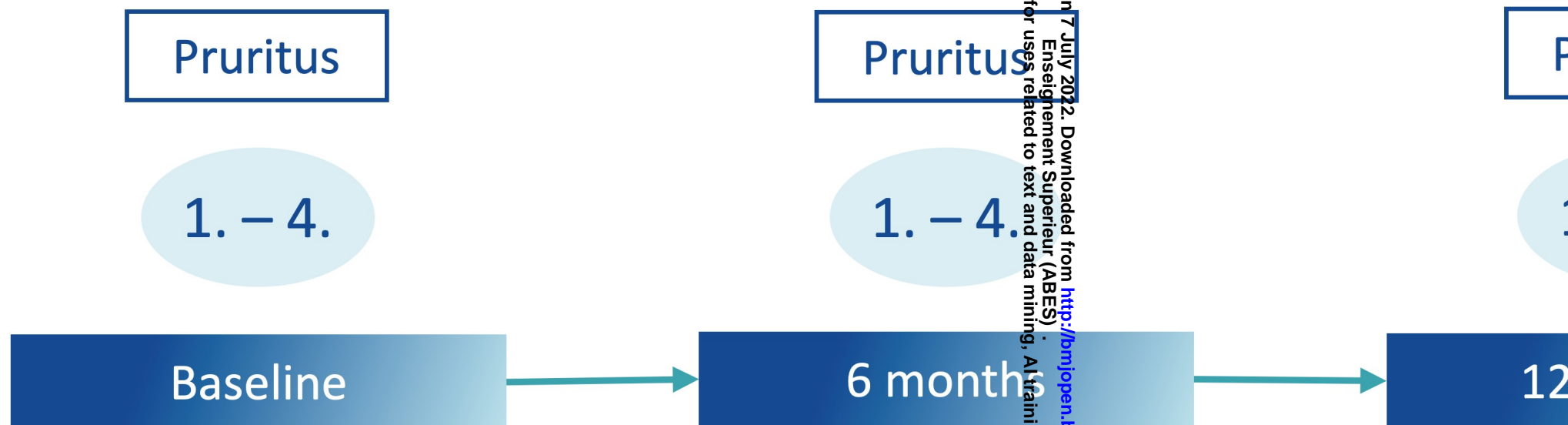
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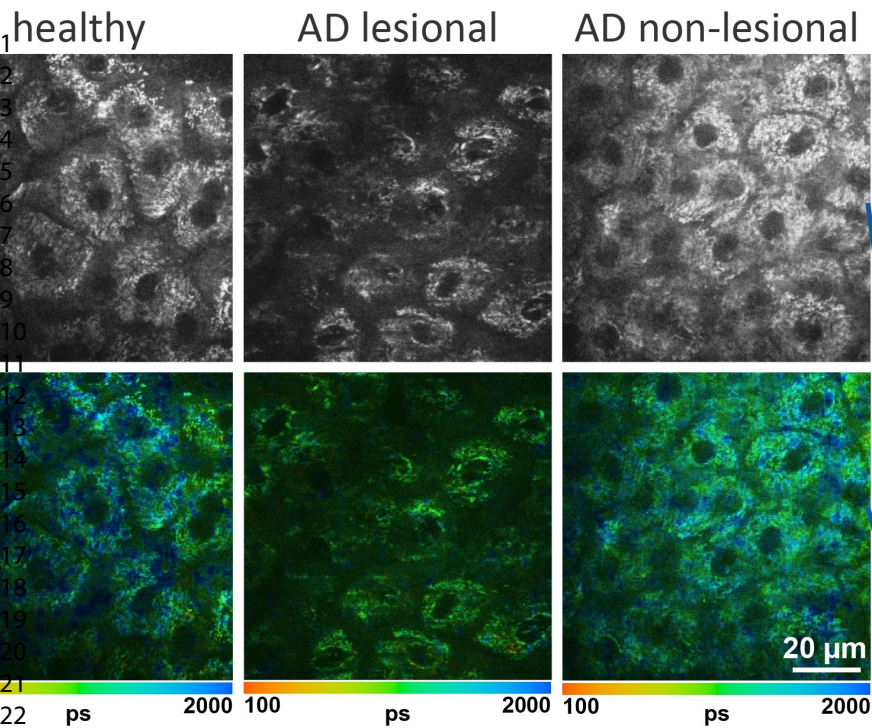


Individuals with CP (n = 120) Healthy Controls (n = 80)

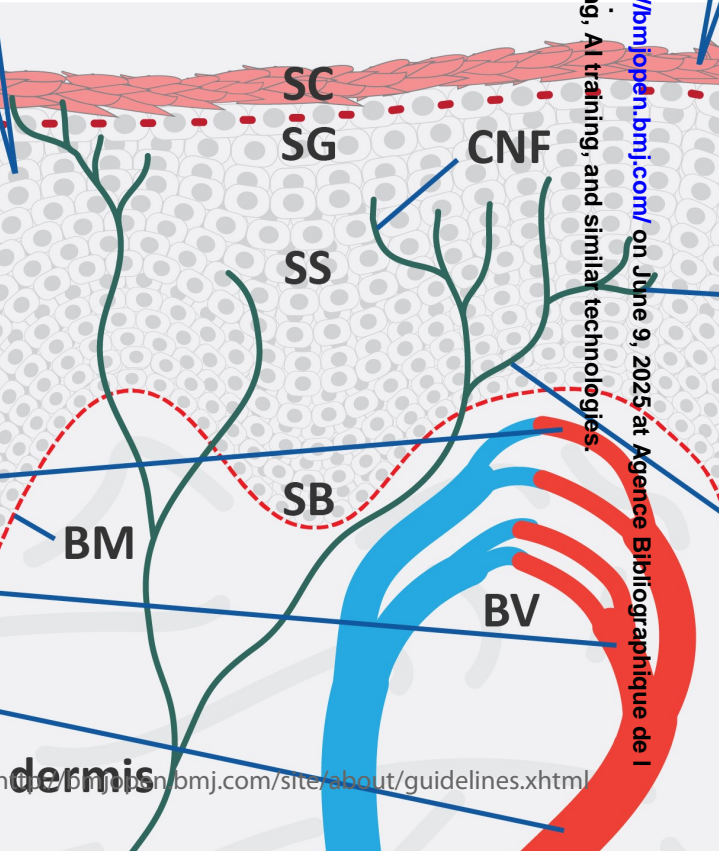
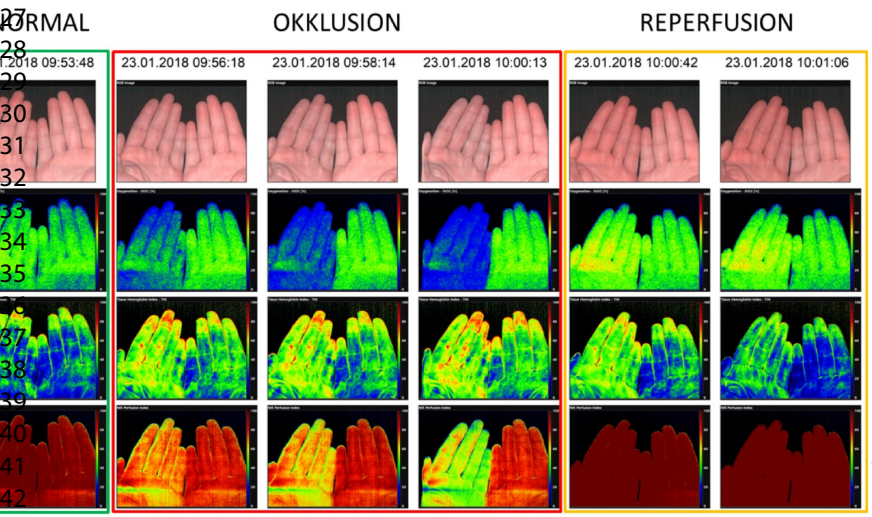


1. Psychosocial aspects (Questionnaire)
2. Neurological analysis (QST, Histology)
3. Skin barrier and metabolism (MPT-FLIM, AFM)
4. Pruritogens (blood analysis)

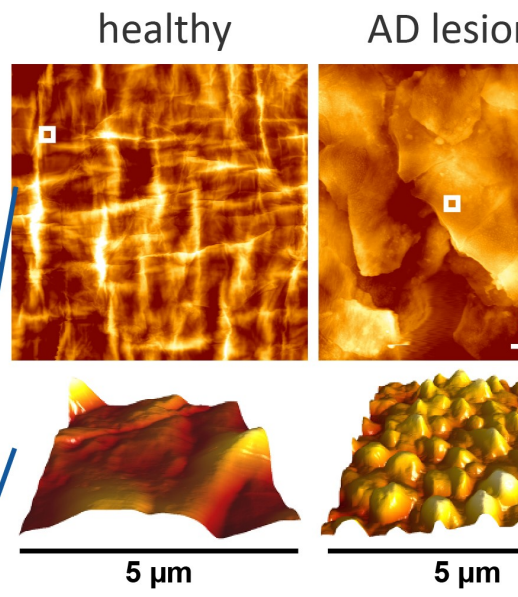
MPT-FLIM



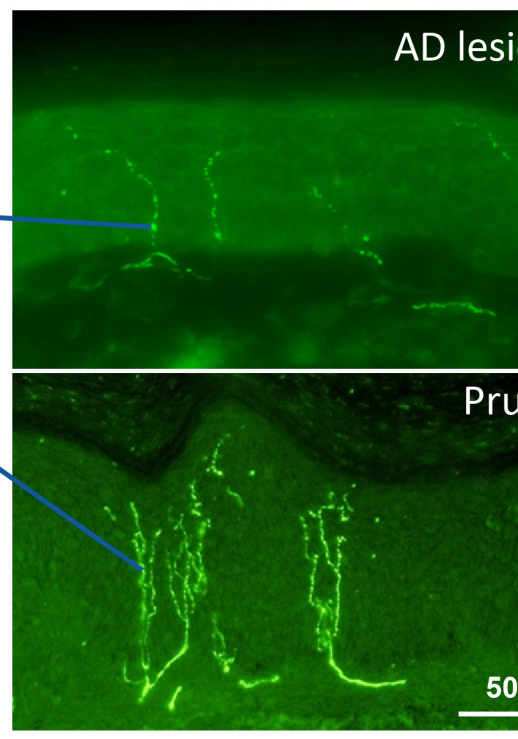
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AFM



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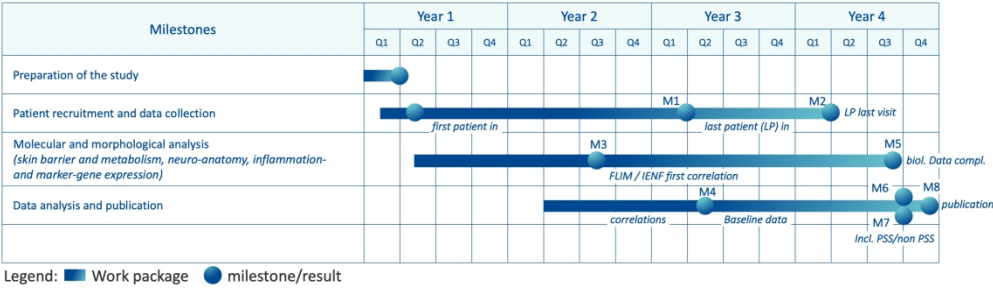


Figure 5: Time table and milestones of project 4

M1. Pruritus/psychosocial questionnaires and clinical data at baseline completed (month 27)

M2. Last patient, last visit; questionnaires and clinical data assessments completed (month 39)

M3. First correlations of collected FLIM / IENF data (month 20)

M4. First correlations of biological and clinical baseline data (month 29)

M5. Biological data assessment completed (month 46)

M6. Correlation of questionnaires, clinical and biological data completed (month 46)

M7. Stratification of patients into PSS/non-PSS and correlation of data at month 12 (month 46)

M8. Publication (month 48)

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