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Study protocol: an observational study of distress, immune function and persistent pain in HIV

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ABSTRACT

Introduction Many people with HIV report both distress and pain. The relationship between distress and pain is bidirectional, but the mechanisms by which distress exacerbates pain are unclear. The inflammatory response to challenge (inflammatory reactivity, IR) may be a partial mediator, given that neuroimmune interactions provide a substrate for IR to also influence neurological reactivity and, thus, pain-related neural signalling. This prospective, observational, case–control study will characterise the relationships between distress, IR, pain-related signalling as captured by induced secondary hyperalgesia (SH), and pain, in people with HIV who report persistent pain (PP) (cases) or no pain (controls).

Methods and analysis One hundred people with suppressed HIV, reporting either PP or no pain, will be assessed two or four times over 6 months. The primary outcomes are distress (Hopkins 25-item symptom checklist), IR (multiplex assay after LPS challenge), and PP (Brief Pain Inventory), assessed at the baseline timepoint, although each will also be assessed at follow-up time points. Induced SH will be assessed in a subsample of 60 participants (baseline timepoint only). To test the hypothesis that IR partly mediates the relationship between distress and pain, mediation analysis will use the baseline data from the PP group to estimate direct and indirect contributions of distress and IR to pain. To test the hypothesis that IR is positively associated with SH, data from the subsample will be analysed with generalised mixed effects models to estimate the association between IR and group membership, with SH as the dependent variable.

Ethics and dissemination Information obtained from this study will be published in peer-reviewed journals and presented at scientific meetings. The study has been approved by the Human Research Ethics Committee of the University of Cape Town (approval number: 764/2019) and the City of Cape Town (ref: 24699).

Trial registration number NCT04757987.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Well-characterised sample with pain-free comparison group matched for sex and age (within 5 years).
⇒ Combines observational outcomes (questionnaires and blood samples) with experimental challenge, to model responsiveness to both immune and neural challenge on a background psychosocial distress status.
⇒ Uses a multi-system approach to probe mechanisms of persistent pain from a biopsychosocial framework that acknowledges multidimensional contributors and sequalae, in line with the International Association for the Study of Pain's updated definition of pain.
⇒ Possible directional or causative features of the associations cannot be elucidated using this cross-sectional design, although the longitudinal aspect will support hypotheses for later formal study.
⇒ Presents a novel opportunity to clarify the intra-individual variations in the outcomes and the inter-individual variance in inflammatory reactivity in people with HIV and good disease control.

BACKGROUND AND RATIONALE

Distress and pain are common and problematic for many people living with HIV (PWH). Approximately 54%–85% of PWH report persistent pain (PP),[1] and this pain is frequently linked to reduced quality of life,[2] worse mental health,[3–6] and worse daily functioning.[7] This complex impact of pain lines up with its biopsychosocial aetiology. The International Association for the Study of Pain recently revised its definition of pain to distinguish pain from the sensory neural activity that is commonly called nociception, and to emphasise that pain is a personal experience that is influenced by biological, psychological and social factors.[8] This biopsychosocial picture is reflected in PWH, who also have higher rates of distress, including internalised stigma, depression, post-traumatic stress disorder and loneliness, than people without HIV.[9–15] Here, we use 'distress' to capture negative affect—for example, symptoms of anxiety and depression, including at subthreshold levels for clinically significant mental illness, acknowledging that psychological processes are already relevant to more health outcomes (including pain) at subthreshold levels.[16]

Distress and pain have a bidirectional relationship. The onset of a pain condition certainly creates and intensifies distress.[16] In addition, distress contributes to pain and its persistence, and treating the distress has been shown to improve pain.[1 17] In PWH, a broad range of distress-related constructs and pathologies (e.g., depression, post-traumatic stress disorder, and psychosocial distress) correlate with pain[1 5 18–20]; indeed, many PWH explicitly identify that psychosocial factors strongly influence their pain.[7 21 22] In South Africa, which is the location of this study, a randomised treatment trial found that just infrequent contact with an empathetic assessor was associated with improved pain outcomes in PWH.[23] Empathetic interactions may foster positive emotions and provide a supportive context for adaptive coping that broadens a person's repertoire of responses to difficult circumstances, increases sense of control, and reduces fear, thus ameliorating distress.[24–26] However, the question remains: through what mechanisms would diminishing distress diminish pain?

Subtle inflammatory changes are thought to be an important mechanism in PP.[27] Importantly, distress is associated with subtle changes in the inflammatory system; particularly, an increased responsiveness at the lower end of the inflammatory response range—a kind of inflammatory 'priming'.[28] In PWH, distress is positively associated with levels of the pro-inflammatory cytokine interleukin (IL)-6.[29] In the general population, anxiety disorders—most markedly, post-traumatic stress disorder—are also associated with elevated levels of IL-6, IL-1β, tumor necrosis factor-alpha and interferon-gamma.[30–32] Loneliness and social isolation have also been linked to increased inflammatory reactivity (IR, inflammatory response to challenge), although results are conflicting in meta-analysis.[33] Importantly, the magnitude of this inflammatory activity is smaller than the pronounced inflammation seen to an infective antigen, or than what would conventionally be called low-grade inflammation; the levels of certain pro-inflammatory cytokines are elevated, but only to a small extent.

This subtle elevation is thought to indicate the potential for heightened immune signalling that would support unfavourable health outcomes after a real-life challenge such as physical or psychosocial trauma—that is, heightened IR. Others have warned that an individual's idiosyncratic response to stressful circumstances may be an important determinant of physiological response,[34] so this subtly pro-inflammatory state may represent an individual-level health risk. To capture IR, it is sensible to measure not only resting levels of various inflammatory mediators, but also the inflammatory response to an actual challenge. Experimental immune challenge elicits greater pro-inflammatory cytokine responses in people who report greater distress.[35 36] In people with complex chronic pain, production of IL-6 and IL-1β in response to in vitro immune challenge predicts the number of sites in which they report clinical pain.[37] An important pain-supporting process is central sensitisation—an 'increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input'. Central sensitisation likely includes both homotopic (same-pathway) and heterotopic (cross-pathway) facilitation of neural activity. The heterotopic component can be indexed using experimental secondary hyperalgesia (SH), which involves over-stimulating an area of skin and testing for increased pain on the application of a stimulus that normally provokes pain in an area adjacent to the over-stimulation.[38 39] Enhanced central sensitisation has been captured after *in vivo* immune challenge, using the SH model: in healthy humans, immune challenge both lowers pain thresholds and exacerbates induced SH.[40 41] Therefore, induced SH is a relevant model of heterotopic facilitation, which is a component of central sensitisation and may support the persistence and spread of pain.

That IR represents vulnerability to pain is consistent with the evidence that neuroimmune interactions contribute to PP states, likely via both peripheral[37] and central[42–44] mechanisms, including in HIV.[45] Interestingly, there may be unique features to inflammatory priming in PWH, given that the HIV envelope protein gp120 is capable of activating the inflammatory glial cells and increasing pain,[46] and that plasma levels of lipopolysaccharide (LPS), a known immune stimulant that induces central sensitisation,[40] remain elevated even after viral suppression.[47 48] The immune challenge approach aims to study the consequences of real-life events (such as psychosocial or physical trauma) to which an organism must respond to restore homoeostasis. Here, we use an *in vitro* immune challenge. A similar approach can be used to challenge the neural system: here, we use an *in vivo* neural challenge (100 Hz electrical stimulation) to probe SH, which models a component of central nervous system processes that may represent vulnerability to PP.

In this study, we aim to take the first steps towards characterising the relationships between psychosocial distress (as indexed by the Hopkins 25-item symptom checklist, which includes symptoms of depression and anxiety), IR (i.e., stronger innate immune response to challenge), experimentally induced SH, and PP in PWH. The study's central hypothesis is that distress is associated with greater IR and therefore greater pain in PWH (figure 1). We hypothesise that (1) part of the relationship between distress and pain is mediated by IR and (2) IR is positively associated with SH. The conceptual framework shown in figure 1 places SH as a potential mediator between IR and pain, but analysis of such mediation would be an additional, exploratory step.

METHODS

Study overview

Figure 2 provides an overview of the study's structure. Consenting adults with HIV and with recent viral load test showing <50 copies/mL blood will be enrolled into two groups (pain-free: reporting no pain; PP: reporting

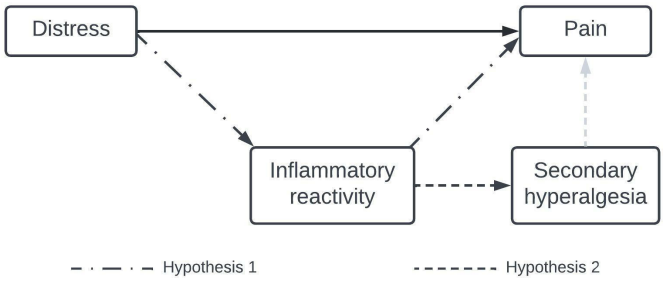

Figure 1 Conceptual diagram. Hypothesis 1 is that inflammatory reactivity (IR) statistically mediates part of the relationship between distress (as indexed by the Hopkins Symptom Checklist) and pain (Brief Pain Inventory). Hypothesis 2 is that IR is positively associated with secondary hyperalgesia (SH). Note that, although the conceptual diagram indicates directional relationships, the cross-sectional nature of the data precludes causative conclusions. Grey colour indicates a relationship not directly tested in this study. Distress could increase IR via the HPA axis and/or the sympathetic nervous system. Distress is linked to a loss of glucocorticoid-mediated constraint of inflammation (usually maintained by the HPA axis), as well as sympathetically mediated release of pro-inflammatory monocytes from lymphoid tissues, and alterations in beta-adrenergic signalling that have a net pro-inflammatory effect, possibly via non-canonical signalling cascades (for review, see Walsh *et al*[28]). Converging evidence supports a role of inflammation in human persistent pain, with the bulk of human studies showing activation of glial cells, including in locations that reflect the somatotopic distribution of pain (for review, see Grace *et al*[27]). Glial cells are a significant (but not the only) source of pro-inflammatory cytokines, placing pro-inflammatory cytokines such as IL-1β and IL-6 as ideal indicators of pain-relevant and SH-relevant IR. Similarly, inflammatory processes potentiate SH, placing the heterotopic facilitation that SH represents as a potential mediator of the pathway between IR and pain, although only the association between IR and SH is a planned formal analysis for this study.

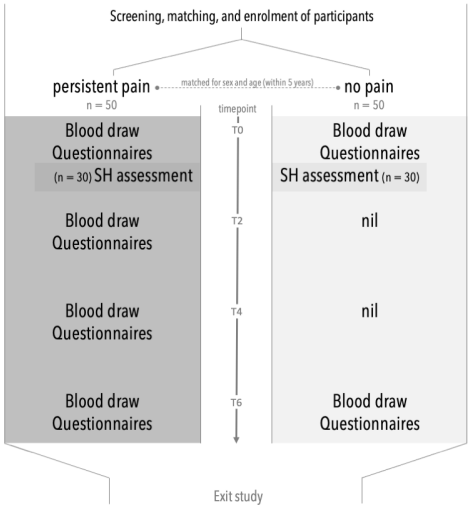

Figure 2 Study overview. Participants are enrolled into two groups and are assessed at T0, T2, T4 and T6 months (participants with persistent pain, n=50), or T0 and T6 months (participants with no pain, n=50), giving blood samples and completing questionnaires at each assessment. A subsample (n=30 per group) also undergoes assessment of induced secondary hyperalgesia (SH) at T0 only.

persistent pain) and undergo assessments over the course of 6 months. Possible assessment time points are at enrolment (T0), and 2 (T2), 4 (T4) and 6 (T6) months later, although the T0 time point is primary and will provide the cross-sectional data to test the two study hypotheses. The pain-free group will have assessment contacts at T0 and T6 only; the PP group will have assessment contacts at T0, T2, T4 and T6. Repeated assessments of the PP group are anticipated to support exploratory examination of the temporal stability of the study variables and relationships between them. In particular, the within-person temporal variation in IR has not been established in this population but will be important for planning future work. The use of more assessments in the PP than in the pain-free group reflects the study's focus on understanding fluctuations in pain. All participants exit the study after T6. The primary outcomes are distress, IR and PP (assessed at every contact), and induced SH in a subsample of participants (assessed at T0 only). The study has been approved by the Human Research Ethics Committee (HREC) of the University of Cape Town (approval number: 764/2019) and the City of Cape Town (ref: 24699), which operates the clinic from which participants will be recruited. Enrolment began in February 2021, and was completed in November 2021. The study is anticipated to run until May 2022.

The study will be conducted in Khayelitsha, South Africa, which is part of the major city of Cape Town. Most of the patients obtaining care at this clinic are isiXhosa-speaking, 'black' African people, and two thirds of them identify as female. National Census data from this area highlight the impacts of poverty and hardship, with 38% unemployment, 78% reporting an annual income less than ZAR3200 (~US$202) per household, and 56% living in informal housing.[49]

Participants

Eligibility screening

The study is open to adults aged 18–65 who have HIV and show evidence of viral suppression (viral load test showing <50 copies/mL within the preceding 3 months), and who self-report as not pregnant. We will define PP as self-reported pain on most days for 3 months or longer,[50] regardless of the intensity of pain. The comparison group will be participants who self-report no pain, with each comparison group participant matched to a participant with PP on the variables of sex and age (within 5 years). Pain status will be ascertained using the following questions, which are modified from the Brief Pain Inventory.[51] 'Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, toothaches)'. (1) 'Do you have pain other than these kinds of pain today?'. Regardless of answer: (2) 'other than those day-to-day kinds of pain, do you have pain in part of your body on most days?'. If NO to both 1 and 2, the person will

be considered pain-free for the purpose of this study. If answers differ to 1 and 2, the person will be considered ineligible for this study. If YES to both 1 and 2, then: (3) 'have you had that pain on most days for more than 3 months?'. If YES to 3, the person will be considered to report PP for the purpose of this study. Exclusion criteria will include: people who are (1) unable to give fully informed consent, and/or (2) unable to engage in the test battery, and/or (3) in need of acute mental healthcare (eg, high suicide risk). Therefore, participants must have no evidence of cognitive impairment, no current substance abuse (alcohol and/or drug) disorder, and no current indications of psychosis, and must be able to communicate in English or isiXhosa. All procedures will be conducted in either English or isiXhosa, as selected by the participant. The informed consent process includes an explain-and-explain-back component during which the clinical research assistant will be trained to detect cognitive difficulties preventing comprehension, and volunteers who show persisting obvious difficulties will not be included in the study. Substance abuse and psychosis will be screened for using questions drawn from the MINI screening tool, followed up with the WHO ASSIST or WHO AUDIT tools (substance abuse) or the psychosis module of the Mini International Neuropsychiatric Interview,[52] as appropriate. Suicidal ideation and severity will be assessed using adapted items from the Mini International Neuropsychiatric Interview,[52] and participants will be excluded and referred for psychiatric services if they meet criteria for high suicide risk at screening. Screening takes about 15 min; informed consent takes about 20 min.

A subsample of participants who have no contraindications to electrical stimulation (eg, electrical implant) and no pain or procedural contraindications (e.g., metal implant or metal-ink tattoo in the relevant forearm, neurological problems such as epilepsy), will be invited to participate in the SH induction and testing procedure, subject to age-matching and sex-matching criteria between groups of the subsample. Participants with pain who take prescribed pain medication will be allowed to continue taking their medication, but no *ad hoc* analgesic medication will be allowed for 24 hours prior to the induction of SH.

Sampling and sample size

Recruitment to the study will use convenience sampling: participants will be enrolled in consecutive order as they screen eligible, but each pain-free participant must match a participant with PP on the characteristics of sex and age (within 5 years). The sample size has been determined pragmatically. We aim to recruit n=50 participants per group, and, of those, to include n=30 per group in the subsample that undergoes SH induction, acknowledging that some participants may not be eligible (eg, if they have metal or electrical implants, forearm tattoos, etc. that render electrical stimulation unsafe) or unwilling to undergo induction of SH. Data from previous studies have found between-group differences in IR of d=0.6–2.29 when groups differ by pain status,[37 42 53] and between-group differences in SH of d=1.79–2.73 between conditions/groups defined by IR,[54] cognitive-behavioural training,[55] and negative affect.[56] The planned sample size of 50 participants/group will allow approximately 70% power to detect a 'medium' effect size of d=0.5 at baseline.

Compensation and withdrawal

Participants will be compensated for each assessment visit. The compensation amount is ZAR150 (~US$10.50) per questionnaire-and-blood-draw assessment visit, and ZAR150 for each SH induction, to account for travel costs, time and inconvenience. Participants who communicate with the research team to withdraw from the study will be given the option to withdraw all their data from the study, including those previously collected, or to allow the data previously collected to be retained and used.

Variables

Table 1 lists the variables and measures to be used, and the number of participants to be assessed, at each time point. Note that several covariates have been identified and will be accounted for in the planned analysis (see note in table 1). Outcomes 9–12 are included due to hypothesised relationships with distress and/or pain. All communication/assessment materials that do not yet exist in isiXhosa (e.g., explanatory materials for SH induction) have been written in English, translated to isiXhosa, and back-translated to ensure consistent meaning (semantic equivalence).[57]

Measure for IR

The immune response of peripheral blood cells to challenge has shown concordance with the response of central immune cell in both preclinical and clinical studies on animal models of pain—a finding that aligns with the recognised bidirectional communication between peripheral and brain/spinal immune cells.[58 59] Clinically, this responsiveness to stimulation (IR) distinguishes people with PP from healthy controls[36 60–62] and predicts the number of extra-pelvic pain sites in people with painful bladder syndrome.[37] Together, these data suggest that peripheral blood cell responsiveness to stimulation is a reasonable and achievable surrogate for immune responsiveness in the central nervous system, providing a 'window' into central neuroimmune synapse reactivity in the current absence of safe direct access to the central nervous system in humans. Therefore, the IR outcome—proteins identified in supernatant after in vitro stimulation of peripheral blood—is not exclusively reflective of peripheral processes. Rather, it is the common result of numerous processes, including both central and peripheral or systemic contributors. We will use the closed TruCulture system (MyriadRBM) for in vitro stimulation of whole blood with the TLR4 agonist LPS and measure IL-1β and IL-6 in the supernatant.[37 59–61] These two, typically pro-inflammatory, cytokines are of particular interest given the quantity of data linking them to both distress[32 35 63–67] (including in PWH, for IL-6[29]) and pain[37] (including in PWH, for IL-1β[45 46]). Additionally, experimentally induced changes in SH and pain threshold are positively associated with IL-6,[40 41] which may be elevated in PWH compared with HIV-negative peers.[47 48]

Table 1 Main outcomes and measures and participant numbers

No.	Variable and measure	Further information	Time (months) 0	2	4	6
1	**Psychosocial distress**[*δ]**:** 1. 25-item Hopkins Symptom Checklist	1. Used with PWH and people without HIV in South Africa[77 78]	✓	✓	✓	✓
2	2. SRQ-20[76] (supplementary)	2. Endorsed by the WHO; intended specifically for developing countries. Has been used in South Africa with an 'at risk' cut-off score of ≥8[79 80]	✓	✓	✓	✓
3	**Pain:** Brief Pain Inventory[51]: Pain Severity/Intensity subscales; body map	Validated in Xhosa-speaking populations in SA.[81] Body map adapted by dividing the body into 18 different regions. Rating scales offered with supportive hard-copy vertical Visual Analogue Scales (preferable for low literacy participants),[82] allowing participants to select rating using a pen. Ratings are recorded directly into electronic copy by the clinical research assistant	✓	✓	✓	✓
4	**Inflammatory reactivity:** IL-6; IL-1β	Measured in supernatant after whole blood stimulation with LPS. Blood draw required	✓	✓	✓	✓
5	**CD4 count***: nadir and current	Obtained from medical records (nadir, T0) and direct testing (current, T6)				✓
6	**Viraemia***: viral load	Viral load <50 copies/mL indicates viral suppression; test must have been performed within 3 months before the baseline time point and treatment adherence must be good for this result to be acceptable	✓			
7	**ART and other medication***: Self-report and medical history (record review)	Participants will be asked about and records reviewed for all current medications, including antiretroviral treatment and analgesic medication	✓	✓	✓	✓
8	**Coinfections***: Self-report and medical history (record review)	Participants will be asked about and records reviewed for illness in the preceding 3 months, key signs and symptoms of tuberculosis, and any other co-infections	✓	✓	✓	✓
9	**Childhood trauma***: Childhood Trauma Questionnaire—SF[83 84]	Has been used in various groups in South Africa[80 85]	✓			
10	**Stressful life events***: Brugha Recent Life Events Questionnaire[86]	21-items; asks about life events in preceding 12 months and ongoing effects on participant's life. History of stressful/traumatic life events is positively associated with surface area of induced SH[55 56]	✓	✓	✓	✓
11	**Social support***: Medical Outcomes Study Social Support Survey	Has strong psychometric properties[87] and has been used in PWH in South Africa[88 89]	✓	✓	✓	✓
12	**Stigma:** HIV/AIDS Stigma Instrument—PLWA (HASI-P)[90]	Developed and validated in several African countries, including SA. Assesses domains of verbal abuse, negative self-perception, healthcare neglect, social isolation, fear of contagion and workplace stigma, specifically related to HIV status. Includes 33 items	✓	✓	✓	✓
13 14	**Induced secondary hyperalgesia†:** 1. Surface area of skin affected by SH 2. Self-report of pain	Quantitative sensory testing is used to assess SH before and after experimental induction. Stimulation modalities: punctate mechanical stimulation, single electrical stimulation, and soft brush stroke. Self-report on vertical Visual Analogue Scale (preferable for low literacy participants)[82] with anchors identical to intensity scale of the BPI	✓			
15	**Temporal summation†**	Assessed before experimental induction of secondary hyperalgesia (n=60)	✓			
Participants in each phase						
Pain-free		n=50 recruited at baseline	50	0	0	50
Reporting persistent pain		Based on reporting persistent pain at baseline (outcome 3 above). Participants who meet this criterion but report no pain at follow-up time points will be retained in the study. n=50 recruited at baseline	50	50	50	50

Time is indicated in months after recruitment and baseline assessment.
*Anticipated covariates for analyses.
†Only applicable to n=30 per group.
IL, interleukin; LPS, lipopolysaccharide; PWH, people living with HIV; SH, secondary hyperalgesia; SRQ, Self-Reporting Questionnaire.

Interestingly, data on the relationship between IL-1β and distress are rather variable, which could be due to sex differences,[68] and certainly piques our interest. We plan to also use a larger, multiplex panel to comprehensively capture inflammatory response to challenge in this study, but here we commit to hypothesising a role for IL-1β and IL-6 because they represent the most likely overlap between the distress and pain.

Method and measure for induced secondary hyperalgesia and temporal summation variables

High-frequency electrical stimulation[69–73] is an established method of inducing SH to provide a human surrogate model of the spinal, heterotopic, long-term potentiation thought to underlie the persistence and amplification of pain-related neural signalling.[72] The induction of SH will consist of electrical stimulation at 10 times the participant's detection threshold, delivered in five trains of 100 Hz stimulation (400 V, 2000 µs pulse width), separated by 9 s breaks. To reduce confounding by venepuncture-induced sensitisation, the induction will be applied to the forearm that was not used for the blood draw, or on the forearm that was used for the blood draw but at least 7 days after the blood draw. Participants will rate each train on a modified, vertical Visual Analogue Scale that will be provided on a touch screen, by using a stylus pen to swipe across the scale to mark their rating. Scale anchors will be: bottom=no pain; top=pain as bad as you can imagine. To facilitate insight into the scale, each successful swipe will trigger visual feedback on the rating provided, in the form of the scale bar filling up with red colour to the level of the swipe. Quantitative sensory testing (QST) will be used to assess the magnitude and surface area of SH. QST is performed before and after the induction (within-subject *time* control)[71 72 74] to a total of four repeated measures on each site.

Temporal summation (outcome 15, pre-induction testing only) is assessed before the induction only, using self-report to stimulation with a 256 mN pinprick stimulator. The participant-reported rating of a single stimulation is subtracted from the rating of the 10th of a series of ten stimulations to obtain outcome 15 for each participant.

The *surface area of experimentally induced SH* (outcome 13) is assessed in only the follow-up period, using a 128 mN Von Frey filament in a screening test and the eight radial lines method.[75] First, the participant is asked whether they perceive a distinct difference in sensation between a 128 mN Von Frey filament stimulus just distal to the cubital fossa and the equivalent stimulus at the most distal area of skin that is just proximal to the electrode. Second, the same question is used in a comparison between stimuli at the volar wrist and just distal to the electrode. A response of 'yes' to either screening step (indicating distinct difference in sensation, interpreted as a blunt indication of likely SH) initiates the radial lines method to estimate the borders of the area of higher sensitivity. The eight points of transition are used to compute an estimation of the surface area of skin showing increased response to this punctate mechanical stimulation at post-induction assessment. Screening procedures that do not result in a 'yes' response (ie, only 'no' or unclear responses) result in a surface area estimate of 0 cm^2. We include the screening test in response to our experience that some participants report very subtle transitions (that may not reflect actual hyperalgesia) as a boundary, and we wish to confirm that there is an actual difference in sensation to avoid reporting a surface area value for SH that does not exist. Consequently, our chosen bias is towards false negatives, rather than false positives. The surface area values will be plotted, and the within-participant area under the curve will be computed and used in the analysis.

The *self-reported pain to punctate mechanical stimulation* (outcome 14) is computed using the mean of ratings to the two weights of blunt pinpricks at each time point. The within-participant rating at each follow-up time point will be expressed as a percentage of the within-participant mean rating from the baseline time point. Follow-up percentages will then be plotted, and the within-participant area under the curve for each outcome computed and used in the analysis.

Procedures

All study procedures will be conducted in a private and quiet room and will be preceded by verbal confirmation of the previously granted written informed consent.

Baseline (T0) assessment contact

Venepuncture will be performed to supply blood samples. A blood sample that is not for immediate stimulation will be drawn after the TruCulture sample. A trained clinical research assistant designated as assessor 1 will administer the battery of self-report questionnaires, starting with the demographics questionnaire. To avoid confounding of blood results by rapid stress responses to any emotionally triggering questionnaire items but to also allow thawing of the TruCulture tube, the blood draw will take place either during or immediately after the completion of the demographics questionnaire. Together, these processes take about 90 min. Participants included in the subsample to undergo SH induction will then undergo that procedure either immediately, or at the soonest possible time point.

Induction of SH (T0, subsample only)

Figure 3 represents the induction and testing procedure described above. This procedure will be conducted in a separate room to the questionnaire and blood draw assessments. QST is performed in the following order: surface

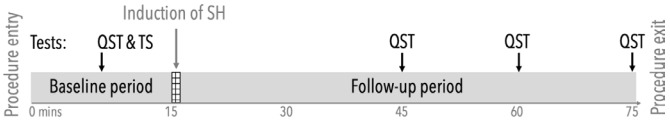

Figure 3 Procedure for experimental induction of secondary hyperalgesia (SH) and obtaining outcomes 13–15. A subsample of participants from each group will undergo quantitative sensory testing (QST) on one forearm, before and after the induction of SH on that forearm (i.e., four repeated measures). Temporal summation (TS, outcome 15) is also assessed, at baseline (i.e., before the induction) only.

area assessment (which takes approximately 5 min), punctate mechanical stimulation (128 mN and 256 mN blunt pinpricks), static light touch (32 mN Von Frey filament), dynamic light touch (brush stroke) and electrical stimulation (single pulse) (which, together, take about 2 min).

Follow-up assessment contacts (T2, T4 and T6)

Brief screening will be used to detect acute psychosis or suicidality, and study procedures stopped to allow referral if either is detected. Venepuncture and the questionnaire battery will be repeated, with the exception of the CTQ (which is not expected to change over time). At T6 only, the venepuncture procedure will include blood draw for CD4 count, taken after other blood samples. Follow-up assessment contacts take about 60 min.

Handling of blood samples

TruCulture samples will be incubated at 37°C for 24 hours, separated using a Seraplas valve filter, and then immediately frozen at −20°C. Unstimulated samples will be drawn into a plain serum tube with clot accelerator (red top), allowed to stand for 30 min, centrifuged at 1800g for 15 min (centrifugation repeated if separation is inadequate), and the serum is moved into a cryovial that is immediately frozen at −20°C. Once fully frozen, all tubes are transported from the research site to institutional storage on frozen gel packs, stored at −20°C again, and then moved to −80°C after 28–33 days, for later batch processing. We will analyse the samples using a multiplex inflammatory panel, but we have prespecified IL-1β and IL-6 levels as being of primary interest. Unused serum/supernatant samples will be retained for a maximum of 15 years to maximise their value for research.

Blood samples for CD4 count will be drawn directly into EDTA tubes and allowed to stand until testing within 72 hours of being drawn. CD4 counts will be performed by the National Health Laboratory Service.

Blinding

The clinical research assistant (assessor 1, who determines participant eligibility, enrols participants, draws blood, and administers questionnaires) and the participants will not be given the specific aims of the study. The consent form states, 'Some people living with HIV also experience distress, and some have pain. The purpose of the research is to understand how this distress can be related to pain and to the body's system that protects you from disease and illness, which is called the immune system'. Assessor 2 (who conducts SH induction and testing) will be blinded to participants' group membership and all questionnaire responses. Assessor 2 undergoes blinding assessment at the completion of the follow-up testing for SH by (1) providing a best guess about the participant's group membership and (2) rating confidence in that best guess, on a Likert scale with options of 'not at all confident', 'not confident', 'neutral', 'confident' and 'extremely confident'. Subsample participants will be blinded to hypotheses associated with the induction and testing of SH. Subsample participants also undergo blinding assessment at the completion of the follow-up testing for SH: the assessor asks each participant what they thought the procedure was about, and uses conservative criteria and exploratory questioning to judge whether participant blinding has been broken. These results will be used in sensitivity analyses, as required, to assess the influence of blinding on the study findings.

Protection of human subjects

Adverse events will be recorded regardless of presumed cause, and reported in all cases. A four-person study steering committee will regularly review information on safety of participants and data, and advise on the need for any amendments to optimise ongoing protection of both.

Data management

Recording and quality control

Participant contact details will be kept electronically under password protection, accessible to only research staff involved in this study, and destroyed on completion of the study. Each participant will be issued with a unique study ID code. Medical record and self-report data will be collected directly into electronic copy wherever possible and coded with the participant's individual study ID. Blood samples will be coded by study ID, study time point, and date and time of the blood draw. Medical record and questionnaire data will be cross-checked for completeness and plausibility (including range checks and comparison of data across related fields) by two independent assessors, omissions/errors addressed, and complete records kept of all additions/alterations made to data. Self-report data from the SH procedure are automatically recorded into soft-copy, and will be reviewed for plausibility using an automated script and visual review of data plots.

Data analysis

Descriptive analysis will summarise measures with mean (SD) or frequency (percent), as appropriate, by group (PP, no-pain) and by time point, with the analyst blinded to group. Longitudinal measures will be represented graphically. We hypothesise that part of the relationship between distress and pain is mediated by IR. Mediation analysis will be applied to estimate the direct and indirect contribution of distress to pain (clinical pain intensity), hypothesised to be mediated by IR, using the baseline data from the PP group. Estimates and 95% bootstrap CIs for direct and indirect effects will be estimated. Secondary analysis will repeat this approach using the number of painful sites as the pain outcome in the same data. Note that the primary mediation analysis will be tested on cross-sectional data because (1) the proposed relationships are thought to be immediate, rather than changes occurring over time; (2) our main interest is in explaining inter-individual differences, rather than intra-individual relationships, and (3) the baseline data will provide the best power, given anticipated attrition. However, the longitudinal data will allow for later, exploratory analysis of the temporal stability of the mediation

model, and longitudinal mediation to capture relationships between the variables within individuals, over time.

We hypothesise that IR is positively associated with SH. To characterise the relationship between IR and SH, data from both groups (PP and no-pain) of the subsample will be used with generalised mixed effects models to estimate the association between IR and group membership with SH surface area/magnitude as the dependent variable. For this, the analyst will be blinded to group. Random effects will be fit as a random intercept for individuals and coefficient estimates (95% CIs) reported. Repeated measures will similarly be modelled through mixed effects linear models with appropriate random effects terms. All models using matched data will use conditional estimates in order to account for the matched design.

Data sharing

Coded data that contain no identifiers will be handled and analysed with the support of a private, password-protected github repository. The final dataset will include demographic, health history, psychological, and physiological (e.g., immunological) data. An important priority when considering sharing of these data is participant confidentiality, particularly considering the stigma and personal social risk associated with HIV+ status in South Africa. If the risk to participants can be eliminated, then:

- The final dataset will be stripped of individual identifiers.
- The final, de-identified dataset will be assessed for opportunities to deductively identify individual participants and additional stripping performed as necessary.
- The data and associated documentation will be made available to qualified users through a data sharing platform approved by the institutional HREC.
- A data-sharing agreement will provide for: (1) a commitment to using the data only for research purposes and not to identify any individual participant; and (2) a commitment to securing the data using appropriate computer technology.

Our intention is to place no time limit on the storage of the data: other research indicates that long-term retention of data fosters the progress of science and thus maximises the benefits of research studies such as this one. However, the period of data retention may be necessarily limited by pragmatic concerns such as cost and appropriate supervision—especially if it is ethically inappropriate to release the data to an open repository, which we anticipate as possible. We commit to retaining the data for at least 15 years, during which time VJM will be primarily responsible for the safety, security and confidentiality of the data. In the event of VJM being unable to meet this responsibility, the research team will delegate this responsibility to another member.

Patient and public involvement

There was no patient or public involvement in the design of this study. Our intention is to partner with a community advisory board to determine the most suitable approach to disseminating the results of the research.

Time frame

We began screening and enrolment in January 2021, and we completed enrolment by December 2021, with follow-up assessments running until May 2022.

Limitations

The current study includes participants regardless of pain intensity. The BPI question used to group participants specifically excludes ‘everyday pains’, reducing the risk of including someone who has clinically insignificant pain, but it is likely that certain participants enrolled in the PP group will have pain of reasonably low intensity. Pain *intensity* is a poor indicator of impact on life participation; pain that has an impact seems worthy of attention. However, it may be useful to investigate the same hypotheses in participants who have high-impact pain.

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Contributors VJM conceived the study. All authors contributed to the design of the study. VJM, NM, LM and GJB are involved in collecting data for the study. VJM drafted the protocol manuscript. NM, LM, ML, GJB, MH, JGP, RP, AS, RE and JAJ read, critically revised and approved the manuscript.

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