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CXCR4 PET/CT for detecting inflammatory activity in systemic sclerosis

Protocol Version : Version 4

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Content

1. General information

1.1. Institutions involved and responsible persons

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2. Synopse

TITLE	CXCR4 PET/CT for detecting inflammatory activity in systemic sclerosis
DATE VERSION	09.FEB. 2022 Version 4
AIMS	As pilot study, visualization of inflammatory activity in systemic sclerosis using CXCR4-PET/CT.
RATIONALE FOR THE STUDY	Systemic sclerosis (SSc) is a heterogeneous disease characterized by inflammatory, vasculopathic, and fibrotic changes. Initially, inflammatory changes usually occur, which progress to fibrosis. Various organ systems such as the lungs, skin, heart, and gastrointestinal tract are affected. Early detection of inflammatory activity is therefore important in order to prevent consequential damage, in particular irreversible fibrosis. Since the inflammatory foci can spread throughout the entire body, there is a need to be able to detect inflammatory activity over a large area. The 68Ga-Pentixafor-based imaging of the CXCR4 chemokine receptor, which is expressed on immune system cells such as lymphocytes and macrophages, offers a useful approach here, as it allows specific inflammatory cells to be visualized that migrate to inflammatory lesions via the corresponding ligand (CXCL12) and are involved in the pathogenesis of SSc. To date, only chest CT has been used to diagnose and monitor the progression of pulmonary fibrosis in SSc. This non-functional imaging technique makes it virtually impossible to assess inflammatory activity.
NUMBER OF PARTICIPANTS	12 patients (pilotstudy)
STUDY POPULATION	Patients with SSc, with a disease duration of less than 5 years and with interstitial lung disease (ILD)
STUDY DESIGN	Explorative, prospective pilotstudy, not blinded, not randomized
PRIMARY ENDPOINT	Measurement of the maximum measurable 68Ga-pentixafor tracer activity in CXCR4-PET/CT
DURATION	Ca. 1 year
NUMBER OF CENTERS	Monocentric at the University Hospital Würzburg

3. Background

Interstitial lung disease (ILD) is the most common cause of death in patients with systemic sclerosis (SSc). With regard to the pathogenesis of ILD, but also of other manifestations of SSc, it is postulated that leukocytes initially migrate into the tissue and cause an inflammatory reaction, which subsequently leads to fibrosis. Active inflammation can be treated much more effectively with medication than fibrosis.
The distinction between fibrosis that has already occurred, i.e., is usually irreversible, and the active stage with inflammation is therefore of great therapeutic importance.
Stromal cell-derived factor 1 (SDF-1 or synonymously CXCL12) and its receptor C-X-C motif chemokine receptor 4 (CXCR4) are involved in angiogenesis and the mediation of leukocyte migration, which is an important pathomechanism in SSc. Consistent with this, high SDF-1 and CXCR4 expression is found in the skin of SSc patients, especially in the early stages of the disease, which decreases with increasing disease duration (Cipriani et al. 2006).
SSc patients may have antibodies against CXCR4. Higher antibody titers are associated with more severe lung involvement and poorer lung function. CXCR4-positive mononuclear cells from peripheral blood are found to be reduced in SSc (Weigold et al. 2018).
The consideration of the CXCR4/CXCL12 axis in SSc therefore appears promising.
The Clinic and Polyclinic for Nuclear Medicine at Würzburg University Hospital has a manufacturing license (issued by the Regional Council of Upper Franconia) for the production of 68Ga-Pentixafor for imaging the chemokine receptor CXCR4. CXCR4 is expressed by many cells of the hematopoietic system, such as stem cells, neutrophil granulocytes, monocytes, T lymphocytes, B lymphocytes, pre-B cells, dendritic cells, and macrophages. It is also detectable on most malignant cells.
During inflammatory processes, CXCL12, the ligand of CXCR4, is upregulated in the affected tissue. The chemotactic effect of this ligand leads to the migration of CXCR4-positive cells into the inflamed area, which can be visualized in PET using the CXCR4-specific tracer 68Ga-Pentixafor. A significant advantage over the previous standard tracer F-18 deoxyglucose is its independence from glucose metabolism.
Based on experience gained in the use of CXCR4-PET/CT in malignant diseases in studies conducted at the University Hospital of Würzburg, it can be assumed that this new tracer should enable sensitive visualization of inflammatory processes in SSc and thus functional imaging. This assumption is supported by the fact that CXCR4 can be visualized using PET/CT in idiopathic pulmonary fibrosis (IPF) – a clinical picture that is very similar to ILD in SSc in terms of clinical presentation, prognosis, and treatment (Derlin et al. 2021).
To date, only non-functional cross-sectional imaging using chest CT, which is routinely performed every 1-2 years, has been established for the diagnosis and monitoring of pulmonary fibrosis in SSc.

4. Aims of the study

Testing the hypothesis that tracer activity in 68Ga-pentixafor PET/CT correlates with disease activity in the lungs (heart, and skin) and that activity is associated with disease progression, i.e., worsening fibrosis in the corresponding organ.

Another objective of the study is to characterize CXCR4 expression in monocytes, granulocytes, and lymphocytes in peripheral blood using flow cytometry and, if necessary, cell culture analysis, as well as to measure CXCL12 and CXCR4 antibodies in peripheral blood. The leukocyte populations mentioned above are particularly important for the pathogenesis of inflammatory changes in SSc. A correlation with 68Ga-pentixafor uptake will be investigated.
A further objective is to correlate 68Ga-pentixafor uptake with routinely available clinical, imaging, and laboratory parameters.
The aim is to use the data collected to perform a well-founded case number calculation for a subsequent prospective study.

5. Study design and endpoints

5.1. Design

The project is designed as a monocentric, exploratory, and prospective pilot study that is neither blinded nor randomized. The pilot study will take approximately one year to complete. For individual participating patients, participation in the study ends with the planned CXCR4 PET/CT examination. The parameters routinely collected after 6 and 12 months will be included in the evaluation.

5.2. Endpoints

The primary endpoint of the pilot series is to determine the maximum measurable 68Ga-Pentixafor uptake in CXCR4-PET/CT in the area of fibrotic pulmonary changes.

Secondary endpoints:
- Determination of the maximum measurable 68Ga-Pentixafor uptake in CXCR4-PET/CT in the heart
- Correlation of the measured tracer activity with clinical, laboratory, and imaging findings.

The secondary endpoints serve to classify the measured tracer activities should significant fluctuations occur within the group.

6. Course of the study

6.1. Inclusion criteria

- Confirmed diagnosis of SSc based on the 2013 ACR/EULAR criteria
- Disease duration less than 5 years (onset of the first non-Raynaud's symptom within the last 5 years)

- Evidence of ILD in computed tomography
- Patient's ability to understand the information provided
- Patient of legal age
- Exclusion of contraindications for performing a PET/CT examination (see below)

Patients are informed in detail, both in writing and verbally, about the purpose of the examination and the risks of participating in the pilot study. The information is provided with sufficient time for consideration before the planned PET/CT scan. The Declaration of Helsinki and the guidelines of Good Clinical Practice are observed in all respects. Consent to participate in the pilot study and any questions/special circumstances are documented in writing by the patient's signature on the consent form, which must also be signed by the physician providing the information.

6.2. Exclusion criteria

- Following autologous stem cell transplantation
- Presence of pulmonary hypertension
- Contraindications for performing a PET/CT scan
- Pregnancy
- Breastfeeding
- Allergies to 68Ga-Pentixafor
- Patient's inability to understand and give informed consent

6.3. Recruitment and study procedure

Timeline of the pilot study:
a. Screening of patients during their appointment at the rheumatology outpatient clinic
b. Review of inclusion and exclusion criteria
c. Inclusion in the pilot study
d. Determination of baseline parameters:
i. mRSS
ii. Troponin/NT-pro-BNP
iii. Leukocyte differentiation (flow cytometry/cell culture), CXCL12 determination, and CXCR4 antibody determination
iv. Echocardiography
v. Lung function with 6-minute walk test
vi. Collection of indices for probability of progression (analogous to the INBUILD study, Flaherty et al. 2019), extent of lung involvement (according to Goh et al. 2008) and activity index according to EUSTAR (Valentini et al. 2017).
vii. ANA/ENA, fibrillarin, RNA polymerase III, myositis line blot
e. Performance of a CXCR4 PET/CT scan
f. Routine clinical check-ups are performed after 6 and 12 months, including points i.-vi. Follow-up checks of ILD using chest CT are performed after 12 months, in accordance with routine clinical practice.

6.4. PET/CT scanning

The PET/CT examination is performed in accordance with the current guidelines of the German Society of Nuclear Medicine and the European Association of Nuclear Medicine. Patients are informed about the planned examination by the responsible study physician and give their written consent to the examination. The explanatory discussion takes place with sufficient time for consideration before the start of the examination.

Patient preparation

Patients do not need to fast before imaging with the tracer 68Ga-Pentixafor. A venous cannula is inserted to administer the tracer. Before the start of image acquisition, patients should empty their bladder completely so that they do not become restless during the examination due to a possible urge to urinate.
After the examination, patients should drink plenty of fluids and empty their bladder frequently to support the renal excretion of 68Ga-Pentixafor and thus minimize radiation exposure.

Image acquisition

The clinical examinations are performed using a PET/CT hybrid scanner (Siemens mCT, Knoxville, TN). The dose coefficient for 68Ga-Pentixafor is 1.56·10-2 mSv/MBq. The radiation exposure for a PET examination with an application of 100-130 MBq is therefore 2.3 mSv. In comparison, the radiation dose for an adult human from natural and civilizational radiation exposure is approximately 2.1 mSv per year; This means that the examination corresponds approximately to the dose of natural radiation received by an adult in Germany (Federal Office for Radiation Protection, accessed on February 9, 2022).
Image acquisition for 68Ga pentixafor PET takes place 45 minutes after injection. The measurement range extends from the neck to the pelvis. In 3D mode, measurements are taken for 2 minutes per bed position, and image reconstruction is performed using an OSEM algorithm (attenuation-weighted ordered subsets expectation maximization algorithm; 4 iterations, 8 subsets).

Low-dose CT protocol

After PET has been performed, the CT series is acquired immediately afterwards using a Siemens mCT Biograph 64. CT acquisition for PET involves low-dose CT (30 mAseff, 120 kV, 0.5 s/rotation, 5 mm layer thickness, pitch = 0.8). An effective dose of 1.7 mSv is to be expected for a low-dose CT of the thorax (scan length approx. 30 cm). The total additional radiation exposure of the PET/CT for the patient is 4 mSv (calculated with CT-EXPO v2.1).

Image evaluation

All CT and PET examinations are initially evaluated visually to determine the intensity and extent of the inflammatory processes. For semi-quantitative assessment of regional inflammatory activity, regions of interest (ROI) are placed in areas of increased [68Ga]-pentixafor uptake using TrueD software (Siemens Medical Solutions, Knoxville, TN). The SUV(mean/max/peak) is calculated within the ROI according to the formula:

SUV = specific activity concentration (Bq/g) x body weight (in g) / injected activity (Bq). In addition to the SUV, other standard quantification parameters, such as the CXCR4-positive volume or mathematically extracted items (e.g., radiomics parameters, Interview Fusion, Mediso Medical Imaging, Hungary), are determined. In [68Ga]-pentixafor PET/CT, the SUVs are correlated with tracer uptake in the background (e.g., in the liver) and a ratio is formed.

6.5. Blood sampling

A venous cannula is inserted for the application of the tracer (see section 6.4). This cannula is used to collect 20 ml of EDTA blood and 5 ml of serum to determine the leukocyte profile, CXCL12, and CXCR4 antibodies (see section 4).

7. Adverse events and safety

7.1. Adverse events

Adverse events are recorded in the electronic case report form (eCRF) created for each patient (see Chapter 9) and, if clinically relevant, communicated to the patient and appropriate measures initiated. If desired, the patient's family doctor can also be informed. Any necessary follow-up checks or further measures are primarily carried out by the attending physician in the rheumatology outpatient clinic or are initiated by them.

7.2. Safety

A request was submitted to HDI-Gerling to register patient insurance as soon as a final positive vote from the ethics committee is available.

8. Evaluation of data

8.1. Case number estimate

The case number estimate was carried out in collaboration with Dr. Mügge and resulted in a total number of 12 participants. The complete case number estimate is attached to the study protocol.

8.2. Biometric analyses and data evaluation

Due to the number of cases, the statistical evaluation mainly comprises exploratory analysis methods. The primary question (maximum tracer uptake in the ILD area versus normal lung tissue) is evaluated using Kendall's tau correlation coefficient.
If possible, subgroups could be examined in relation to limited vs. extensive ILD (Goh et al. 2008) with regard to stratification analogous to the INBUILD study (Flaherty et al. 2019) and with regard to the EUSTAR activity index (Valentini et al. 2017).

9. Data protection, data storage

Personalized data will be collected as part of the study. External cooperation partners, such as the statistician conducting the analysis, will only receive personal data in pseudonymized form. The data will be anonymized for the purpose of scientific publication in a peer-reviewed journal.
The data will be stored in locked rooms at the rheumatology outpatient clinic (A3, -1, ZIM) and on the UKW servers. At the end of the evaluation, but no later than after 10 years, the data collected as part of the study will be anonymized and personal data will be deleted. Any remaining sample material will be destroyed.
Until the data is anonymized, patient-related data records can be deleted and any samples that may be available can be destroyed if the patient revokes their consent.

All data is subject to the data protection regulations applicable throughout the clinic. Test subject data will not be passed on to third parties. The names of the test subjects and all other confidential information are subject to confidentiality and the provisions of the Federal Data Protection Act (BDSG). The pilot study is for research purposes only and its data will not be used (even in the future) for a consultation evaluation procedure.

All patient-related data is recorded in pseudonymized form, and an Excel-based eCRF is created for each patient. Each patient is uniquely identified by a patient number assigned during registration. Dr. M. Gernert maintains a confidential patient list in which the pseudonyms are linked to the full patient names. The evaluation of the collected image data is stored in SAP as a findings document. The data collected for the evaluation of the pilot series is also stored as a pseudonymized Excel file, which contains only the data and the study code of the respective patient.

The blood samples of the participating patients are generally used directly for analysis and then destroyed. In the event that a new analysis is necessary, which only occurs during the course of the study, part of the blood is frozen and stored in pseudonymized form in our in-house immunological laboratory in the rheumatology department. After completion of the study, but no later than ten years, the blood samples will be destroyed. Only physicians and medical-technical assistants involved in the study have access to the blood samples.

The data protection provisions in accordance with the EU General Data Protection Regulation (GDPR) are complied with. The legal basis for data processing is the consent of the study participants (Art. 6 (1a) and Art. 9 (2a) of the GDPR). Participants may request information about the stored data from the UKW within the framework of the legal requirements; if incorrect personal data is processed, participants have the right to have it corrected. They may request the deletion of personal data or the restriction of processing or data transfer. If these rights are asserted against the UKW, it will be examined whether these rights are subject to any restrictions.

10. Consent

Participation in this study is voluntary; consent can be withdrawn at any time without giving reasons (see also “Data protection”). There is no connection between the treatment of patients and participation in the study.

11. Ethics

The project complies with the Declaration of Helsinki (as of October 2013, Fortaleza, Brazil) and Good Clinical Practice.

The protocol and patient information with consent form will be submitted to the local, independent ethics committee of the Medical Faculty of the University of Würzburg.

12. Publication of data

The results or parts of the results of the study are to be published in peer-reviewed scientific journals in anonymized form.

13. Literatur

Bundesamt für Strahlenschutz, Natürliche Strahlung in Deutschland (letztmalig abgerufen am 9.2.2022): https://www.bfs.de/DE/themen/ion/umwelt/natuerliche-strahlung/natuerliche-strahlung_node.html

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14. Abbreviations

ACR American College of Rheumatology
CT Computed tomography
CXCR4 C-X-C motif chemokine receptor 4
CXCL12 C-X-C motif chemokine 12
eCRF Electronic case report form
EULAR European League against Rheumatism
FDG Fluorine 18 deoxyglucose
ILD Interstitial lung disease
IPF Idiopathic pulmonary fibrosis
PET Positron emission tomography
SSc Systemic sclerosis
SUV Surface under the curve

15. Unterschriften

Unterschriften wissenschaftliche Leitung

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16. Appendix

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Schätzung der Fallzahl für das Forschungsvorhaben
CXCR4 Expression bei Systemischer Sklerose –
Interstitielle Lungenerkrankung (SSc-ILD)
mittels a priori Teststärke-Analyse

In der vorliegenden Studie soll die CXCR4 Expression mittels Positronen-Emissions-Tomografie (PET) bei SSc-ILD untersucht werden.

Zur a priori Berechnung der Stichprobengröße ist es notwendig, den zu erwartenden Effekt zu bestimmen. Laut Studienleiter sind keine vergleichbaren Studien für die Korrelation zwischen CXCR4 PET Signal und dem PET-Uptake in SSc-ILD bekannt. Daher wird auf die Zusammenhänge zwischen CXCR4-PET Signal und Lungenfunktionsparametern bei einer vergleichbaren Erkrankung (idiopathische pulmonale Fibrose) zurückgegriffen.[1] Der klinisch am besten passende Effekt zwischen Lungenfunktionsparametern (sog. FVC, forcierte Vitalkapazität) und CXCR4-PET Signal beträgt r = -0,75.

Der Berechnung der Fallzahl liegen somit folgende Prämissen zugrunde:

- Der Fehler 1. Art (α-Fehler) wird gemäß Konvention auf $\alpha = 0{,}05$ festgelegt, da keine überproportional hohen Kosten für einen Fehler 1. Art zu erwarten sind.
- Der Fehler 2. Art (β-Fehler) wird gemäß Konvention auf $\beta = 0{,}20$ festgelegt, da keine überproportional hohen Kosten für einen Fehler 2. Art zu erwarten sind. Die Power beträgt somit $(1 - \beta) = 0{,}80$.[2]
- Es werden ***zweiseitige Hypothesentests*** zugrunde gelegt.[3]
- Die noch relevante, relative Effektstärke ist $r = -0{,}75$.
- Der zu bestimmende Zusammenhang wird durch die ***Pearson-Korrelationen*** ermittelt. Eine Verletzung der parametrischen Voraussetzungen ist nicht zu erwarten.

Die Berechnung der Teststärke mittels der Standard-Software G*Power 3.1 ergibt eine Stichprobengröße von 11 Personen. Bei Forschungsvorhaben sollte mit einem Ausfall von Studienteilnehmern gerechnet werden. Unter Berücksichtigung dieser generellen Ausfallrate und basierend auf den Erfahrungswerten der Studienleiter sei eine Ausfallquote von 10 % als konservative Schätzung anzunehmen.

Somit ergibt sich eine Gesamtteilnehmerzahl von $N = 12$.

Adelebsen, 29. September 2021

Dr. Dirk Mügge

[1] Derlin et al. (2021). Clinical Molecular Imaging of Pulmonary CXCR4 Expression to Predict Outcome of Pirfenidone Treatment in Idiopathic Pulmonary Fibrosis. *CHEST, 159*(3), 1094-1106. https://doi.org/10.1016/j.chest.2020.08.2043

[2] Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*, 175-191.

[3] Farrokhyar, Forough et al. (2013) Practical Tips for Surgical Research: Why Perform a Priori Sample Size Calculation?, *Canadian Journal of Surgery* 56.3, 207–213.

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