SUBMISSION FORM ROCHE BTS RESEARCH GRANT 2020

Instructions for submission:

1. This grant is aimed to reward a Belgian multi-centric research project.

2. The project may be translational or clinical, with the potential to increase availability of organs for transplantation and/or to improve transplant results in Belgium.

3. The PI should be a BTS member.

4. A summary of the project should be sent by e-mail to the BTS secretary (info@transplant.be) using the present form.

5. The project should be written in English. Use Arial 12 font with single line spacing. Maximum 5 pages A4 format.

6. The project should be structured as follows:

- Aim of the study

- A brief description of the Methods

- Estimate of requirements and available facilities (registry data, research lab, medical imaging…)

7. Deadline for submission is 31st January 2020.

8. The winning project will be selected by the BTS Board.

BELGIAN TRANSPLANTATION SOCIETY

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Participating centers:

1. ULiège – CHU, Liège - investigator: François JOURET
2. UZA, Antwerpen - investigator: Annick MASSART
3. UCL – St-Luc, Bruxelles - investigator: Nada KANAAN
4. VUB, Brussels - investigator: Lissa PIPELEERS
5. UGent, Gent - investigator: Steven VAN LAECKE

Topic: Kidney:

Non-invasive diagnosis of subclinical rejection of kidney allograft using ^{18}F-fluorodeoxy glucose positron emission tomography (^{18}FDG-PET/CT): a Belgian multi-centric validation cohort

Submission box:

1. Aim of the Study

Subclinical rejection (SCR) has been defined as "the documentation by histology of unexpected evidence of acute rejection (AR) in a stable patient"[1]. The diagnosis of SCR is important given that the treatment of subclinical T-cell-mediated AR results in similar long-term graft survival to that in patients without rejection[2]. Surveillance transplant biopsies are, by definition, required for the diagnosis of SCR. Still, only 17% of transplant centers in the United States perform surveillance biopsies in all patients, whereas 62% do not perform surveillance biopsies at all because the incidence of SCR is low[3]. Non-invasive biomarkers may thus be developed as "*rule out*" tests, with the highest negative predictive value, in order to reduce the indiscriminate use of allograft biopsies in stable kidney transplant recipients (KTR)[4,5]. Beside urine-derived and blood-based biomarkers of SCR[6], we have recently reported in a monocentric prospective pilot study that ^{18}F-Fluorodeoxyglucose Positron Emission Tomography coupled with Computed Tomography (^{18}F-FDG PET/CT) non-invasively rules out SCR at ~3 months *post* kidney transplantation (KTx)[7]. This study had been approved by the Institutional Review Board of ULiège Academic Hospital under the number B707201215598. This goes in line with our previous observations supporting the usefulness of ^{18}F-FDG PET/CT in the diagnosis AR in kidney transplant recipients (KTR) presenting with acute kidney injury and suspected AR[8].

In our 92-patient cohort, the ratio between the mean standardized ^{18}F-FDG uptake values (mSUV) of the renal allograft and that of the psoas muscle (used as reference tissue) was significantly higher in the group with biopsy-proven SCR *versus* the group with strictly normal histology. The area under the ROC curve (AUC) reached 0.79, with a negative predictive value of 98% using a mSUV ratio (mSUVR) threshold of 2.4. An external validation of this diagnostic threshold is required. That is the reason why we propose the present Belgian multi-centric study aiming at prospectively testing the yield of ^{18}F-FDG PET/CT in SCR detection at ~3 months *post* KTx. The validation of the use of ^{18}F-FDG PET/CT to detect SCR would be the *first-in-class* validated study using an imaging method to rule out kidney rejection. This pioneering study may directly contribute to decrease the morbidity associated with graft biopsies in KTRs. Also, this study may pave the way for further isotopic explorations of the kidney transplant using a variety of radio-labelled molecules under development. The possibility to "see" early intra-graft modifications makes these icono-markers potentially more advantageous compared to other bio-markers.

2. A brief description of the Methods

The sample size calculation on the basis of a 10% incidence of SCR, with α (risk of type I error) set to 5% and power of 80% (1-β), respectively, estimates that 146 patients are needed to statistically test the diagnostic performance of ^{18}F-FDG PET/CT in SCR detection in stable KTR at ~3 months *post* KTx. The participating centers, i.e. CHU of Liège, UZ Antwerpen, Cliniques Universitaires Saint-Luc, VUB and UGent, perform 60, 50, 60, 30 and 30 surveillance biopsies per year, respectively. These numbers suggest that >200 surveillance biopsies will be done *in*

toto within a year in these centers, which, in turn, suggests that the number needed to test (= 150) can certainly be reached within a 18-month period (taking into account patient's refusal, logistical issues and exclusion criteria (*see infra*)).

The 5 participating centers are equipped with PET/CT machines, which are routinely used and readily available. The cross-calibration between machines is not necessary since the threshold of ^{18}F-FDG uptake in the renal allograft to rule out SCR is based on a ratio *versus* the psoas muscle, i.e. mSUVR of 2.4. Additionally, we will prospectively and independently test the diagnostic yield of the mSUVR kidney/liver in the present study and compare it to the diagnostic performance of mSUVR kidney/psoas. In other fields of Nuclear Medicine, the liver is indeed considered as the best comparator[9].

All adult KTR who undergo surveillance transplant biopsy at ~3 months *post* KTx are eligible for the present study, except pregnant women, women of child-bearing age not using an adequate contraceptive mean and patients with PCR-proven BK nephropathy. Written informed consent will be obtained before PET/CT imaging. Surveillance biopsies will be locally (in each participating center) and centrally (at ULiège CHU) assessed by pathologists blinded to the results of ^{18}F-FDG PET/CT. Banff 2017 classification will be used[10]. Total inflammation (ti) will take into account interstitial inflammation in both non-sclerotic and sclerotic areas. C4d staining will be performed in all cases. PET/CT will be performed with late acquisitions (~180 minutes) after ^{18}F-FDG intravenous injection (~3 Mbq/kg) within a 48-hour period around the biopsy, before any modification of immunosuppressive regimens. The mSUV of kidney cortex will be measured and averaged from 4 volumes of interest (VOI) distributed in the upper (n=2) and lower (n=2) poles, both locally (in each participating centers) and centrally (at ULiège CHU)[11]. The mSUV of kidney will be normalized to the mSUV of psoas muscle (VOI of 20 ml) and the liver (VOI of 20 ml), considered as reference tissues in order to obtain the corresponding mSUVR[12,13]. One-way analysis of variance (ANOVA) and Tukey's range tests will be performed using the Python library SciPy and StatsModels (http://www.scipy.org/) to compare the mSUVRs among histology-categorized groups (i.e. normal histology *versus* borderline histology *versus* SCR *versus* others). Additionally, the correlation between the mSUVR and acute composite Banff scores or ti will be assessed using $R = \frac{\mathrm{cov}(x,y)}{\sigma_x \sigma_y}$. Finally, the receiver operating characteristic (ROC) curve used to extrapolate sensitivity and specificity will be built using Python programming language. The performance of the present validation cohort will be evaluated using the diagnostic threshold derived from the training cohort[7] on the basis of Youden's J-statistics.

3. Estimate of requirements and available facilities (registry data, research lab, medical imaging…)

The cost of one ^{18}F-FDG PET/CT is ~200 euros. Hence, the budget of the present study including 150 ^{18}F-FDG PET/CT imaging will be 30.000 euros. The other costs of the study (data base set-up and collection, statistical analyses, publication fees, etc…) will be contributed by the investigating centers.

All medical, clinical and biological data will be systematically collected (see Table 1).

The participating centers, i.e. CHU of Liège, UZ Antwerpen, Cliniques Universitaires Saint-Luc, VUB and UGent, are equipped with PET/CT machines, which are routinely used and readily available. The cross-calibration between machines is not necessary since the threshold of ^{18}F-FDG uptake in the renal allograft to rule out SCR is based on intra-individual ratios *versus* the psoas muscle or the liver[9].

The study will be submitted for approval by the Institutional Review Board of each participating center. Written/Signed informed consent will be obtained from each patient before inclusion and PET/CT imaging.

References

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3. Mehta R, Cherikh W, Sood P, Hariharan S. Kidney allograft surveillance biopsy practices across US transplant centers: A UNOS survey. Clin Transplant 2017;31.
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8. Lovinfosse P, Weekers L, Bonvoisin C, et al. Fluorodeoxyglucose F(18) Positron Emission Tomography Coupled With Computed Tomography in Suspected Acute Renal Allograft Rejection. Am J Transplant 2016;16:310-6.
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13. Jadoul A, Lovinfosse P, Weekers L, et al. The Uptake of 18F-FDG by Renal Allograft in Kidney Transplant Recipients Is Not Influenced by Renal Function. Clin Nucl Med 2016.

Table 1. Clinical and biological characteristics to be systematically collected

Recipients	Age (years)
	Sex (M/F)
	BMI (kg/m^2)
	PRA max (<5%/5%-85%/>85%)
Donors	Age (years)
	Sexe (M/F)
	Donor type [DBD/DCD/LD]
	BMI (kg/m^2)
Transplantation	Rank (1st/2nd/3rd)
	Cold ischemia time(min)
	HLA mismatches locus A locus B locus DR
	Early graft function (immediate/slow/delayed)
	DSA (none/class I/class II)
	Proteinuria (mg/g creat)
Status at the time of biopsy	Maintenance immunosuppression -CNI (CsA/FK/none) -Antimetabolite (MMF/MPA/AZA/none) -mTOR inhibitor (yes/no) -CS (yes/no)
	Duration of KTx at biopsy (days)
	Creatinine (mg/dL)
	eGFR (ml/min/1.73m^2)

Abbreviations: *AZA, azathioprine; BMI, body mass index; CNI, calcineurin inhibitors; CS, Corticosteroids; CsA, cyclosporin A; DCD, donor after circulatory death; DBD, donor after brain death; FK, tacrolimus; KTx, kidney transplantation; LD, living donor; MMF, mycophenolate mofetyl; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; PRA panel reactive antibody*