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Improving the Diagnostic Performance of ^{18}F-FDG PET/CT in Prosthetic Heart Valve Endocarditis

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Abstract

Background—^{18}F-Fluorodeoxyglucose (FDG) Positron-Emission Tomography/Computed Tomography (PET/CT) was recently introduced as a new tool for the diagnosis of prosthetic heart valve (PV) endocarditis (PVE). Previous studies reporting a modest diagnostic accuracy may have been hampered by unstandardized image acquisition and assessment, as well as several confounders. The aim of this study was to improve the diagnostic performance of FDG PET/CT in patients suspected of PVE by identifying and excluding possible confounders, using both visual and standardized quantitative assessments.

Methods— In this multicentre study, 160 patients with a PV (median age 62 [43-73]; 68% male; 82 mechanical valves; 62 biological; 9 TAVR; 7 other) who underwent FDG PET/CT for suspicion of PVE, as well as 77 patients with a PV (median age 73 [65-77]; 71% male; 26 mechanical valves; 45 biological; 6 TAVR) who underwent FDG PET/CT for other indications (negative control group), were retrospectively included. Their scans were reassessed by two independent observers blinded to all clinical data, both visually and quantitatively on available EARL-standardized reconstructions (European Association of Nuclear Medicine Research Ltd.). Confounders were identified using a logistic regression model, and subsequently excluded.

Results— Visual assessment of FDG PET/CT had a sensitivity/specificity/PPV/NPV for PVE of 74%/91%/89%/78%, respectively. Low inflammatory activity (CRP <40mg/L) at the time of imaging and use of surgical adhesives during PV implantation were significant confounders, while recent valve implantation was not. After exclusion of patients with significant confounders, diagnostic performance values of the visual assessment increased to 91%/95%/95%/91%. As a semi-quantitative measure of FDG uptake, an EARL-standardized SUV_{ratio} of ≥2.0 was a 100% sensitive and 91% specific predictor of PVE.

Conclusions—Both visual and quantitative assessment of FDG PET/CT have a high diagnostic accuracy in patients suspected of PVE. FDG PET/CT should be implemented early in the diagnostic work-up to prevent negative confounding effects of low inflammatory activity (e.g. due to prolonged antibiotic therapy). Recent valve implantation was not a significant predictor of false positive interpretations, but surgical adhesives used during implantation were.

Key Words: Endocarditis; Prosthetic heart valve; Positron emission tomography; Computed tomography; ^{18}F-fluorodeoxyglucose

Clinical Perspective

What is new?

- In this large, multicenter cohort of patients who underwent FDG PET/CT for suspected prosthetic valve endocarditis, two significant confounders were identified which may have affected previous studies and, when corrected for, result in significantly improved diagnostic accuracy.
- Low inflammatory activity at time of imaging causes false negative interpretations, while prior use of surgical adhesives causes false positives.
- Previous studies reported widespread cut-offs for quantified FDG-uptake due to a lack of standardization.
- In this study, a standardized cut-off of >2.0 for the ratio between FDG-uptake around the affected valve and in the blood pool (SUV_{ratio}) was 100% sensitive and 91% specific.

What are the clinical implications?

- FDG PET/CT has a high diagnostic accuracy for prosthetic heart valve endocarditis (PVE) when implemented early in the diagnostic work-up, and can detect PVE even when blood cultures or echocardiography are negative, and before structural damage occurs.
- Additional quantitative analysis improves diagnostic accuracy and inter-observer reliability, and the suggested SUV_{ratio} cut-off of >2.0 is applicable in any center with an EARL-accredited system.
- Prior use of surgical adhesives may cause false positive FDG-uptake and needs to be taken into account.
- Physiological inflammation due to recent valve implantation, however, is not a reason to omit PET/CT imaging.

Introduction

Prosthetic heart valve (PV) endocarditis (PVE) is a life-threatening complication with a 1-year mortality of up to 50% that affects up to 5% of patients per year following valve implantation.[1] Unfortunately, timely diagnosis of PVE before the occurrence of severe complications such as perivalvular abscesses or valve dehiscence, which usually require high-risk reoperation, is difficult. Echocardiography, as one of the mainstays of the modified Duke criteria[2], can only visualize structural damage, and the sensitivity and specificity of these criteria, additionally including microbiological and clinical evidence of infection, are substantially lower in PVE than in native valve endocarditis.[3]

Clinical guidelines[4,5] were recently updated following newly available data on the additional value of ^{18}F-fluorodeoxyglucose (FDG) positron emission/computed tomography (PET/CT) in PVE.[6,7] FDG PET/CT aids in the diagnosis of both intracardiac and extracardiac infectious foci by functional visualisation of inflammation, even before structural damage occurs.[7] However, FDG PET/CT findings have been reported to be influenced by several confounders such as myocardial FDG uptake, low inflammatory activity (e.g. due to prolonged antibiotic therapy), prior use of surgical adhesives and recent valve implantation.[8,9] The first three possible confounders can be mitigated with adequate patient preparation, timely implementation of FDG PET/CT in the diagnostic work-up and evaluation of the surgical report[10], while evidence for the influence of recent valve implantation is scarce and inconsistent.[11]

Besides a visual evaluation, FDG uptake can also be measured (semi-)quantitatively, and potential cut-offs for the maximum measured intensity around a PV (standardized uptake value, SUV_{max}), as well as target-to-background ratios (SUV_{ratio}), have been reported.[12,13] However,

these reported cut-offs vary widely due to differences in calibration between scanners as well as measurement and reconstruction techniques.[10,12–15] Therefore, the European Association of Nuclear Medicine (EANM) Research Ltd. (EARL) has provided a standardized calibration and reconstruction method that is currently applied in over 150 centres in Europe.[16]

The aim of this study was to investigate the diagnostic performance of FDG PET/CT in a large multicentre cohort of patients suspected of PVE by identifying and subsequently excluding potential confounders using both visual and EARL-standardized quantitative assessments. Additionally, a cohort of patients with PVs but without suspicion of PVE, who underwent PET/CT imaging for other (i.e. oncological) indications, were included as negative controls.

Methods

In this multicentre study, patients of 6 cardiothoracic centres in the Netherlands who had one or more prosthetic heart valves *in situ* and underwent FDG PET/CT imaging for any indication, were retrospectively included. The study was approved and informed consent was waived by the local Medical Ethics Committees of all participating centres. The data, analytic methods, and study materials have been made available to other researchers for purposes of reproducing the results or replicating the procedure.[17]

Patient identification and selection

All patients with a PV (including percutaneously implanted valves, but excluding valvuloplasties) who underwent an FDG PET/CT scan between January 1st 2010 and March 31st 2016 were included. For all patients, at least one year of follow-up was available. No distinction was made between scans that were performed after first PV implantations or reimplantations.

Data collection

Demographic and clinical data of the included patients and technical data of the included scans were entered into a collaborative database. To prevent possible confounding by follow-up scans during an active or after a previous endocarditis episode, only the first scan for suspicion of PVE was included in the analysis. No patients had undergone both a scan for suspicion of PVE and one for another (i.e. oncological) indication.

Patient data

Demographic data as well as the type of PV(s) and implantation date(s) were retrieved. In case of multiple replacement surgeries of the same valve, the most recent valve implantation date was used. A history of diabetes and previous endocarditis was also noted.

Echocardiographic findings from the same clinical admission as the FDG PET/CT scan were recorded for the presence of vegetations (per valve), abscesses, fistulas, new dehiscence of the PV or paravalvular leakage. If multiple echocardiograms were available, abnormalities on transoesophageal echocardiography (TOE) were considered leading, and in case of multiple TOEs, the reported presence of abnormal findings on one would overrule their reported absence on another to ensure an accurate reference standard and modified Duke classification.

Evidence of cardiac device or lead infection was recorded, and results of blood cultures were scored as positive if at least one blood culture had been positive directly before or during the clinical admission, and the causative micro-organism was recorded. Results of serology and polymerase chain reaction (PCR) were recorded separately. Based on these data and clinical follow-up, the modified Duke classification[2] was calculated. As a measure of inflammatory activity, C-reactive protein (CRP) and leukocyte levels were recorded if obtained close to the FDG PET/CT scan (max. 7 days before or after the date of the scan).

Scan data

Based on the original clinical question of the FDG PET/CT scan (derived from the report), the indication for imaging was categorized as suspicion of PVE versus other (i.e. oncological) indications.

We recorded the scan date and acquisition protocol, including preparatory measures such as a fasting period of more than 6 hours, a low-carbohydrate diet for at least 24 hours and/or a heparin injection, as well as the administered FDG dose (in MBq), the blood glucose level at time of FDG injection, the time interval of scan acquisition after FDG injection, and whether the scanner was EARL accredited and an EARL-standardized reconstruction was available. In case of missing images or insufficient clinical data for a reliable final diagnosis, the scan was excluded from further analyses.

Patient classification

Patients with an FDG PET/CT scan performed for other (i.e. oncological) indications were considered controls by default since these patients had no clinical suspicion of an infection. In order to ensure a reliable diagnostic accuracy representative of daily clinical practice, these negative controls were primarily used for the evaluation of causes of false-positive FDG PET/CT-scans, and were not included in the analysis on the diagnostic performance of PET in patients suspected of PVE.

For patients who did undergo FDG PET/CT for suspicion of PVE, the final diagnosis of PVE was established through expert consensus based on all available clinical and diagnostic data as well as at least one year of follow-up. The modified Duke criteria were scored as well[2], with the exception that echocardiographic findings unrelated to the prosthetic heart valve were not included in the final diagnosis of PVE (e.g. a vegetation on one of the native valves would not

count as a major criterion in the final Duke classification regarding PVE), and data on minor Duke criteria (e.g. vascular or immunological stigmata) was often missing.

Expert consensus was achieved through a stepwise approach in which patients were discussed by a multidisciplinary group of physicians from the departments of Cardiology, Infectious Diseases, Medical Microbiology, Radiology and Nuclear Medicine (the "Endocarditis Team") when the diagnosis was neither confirmed by surgery/histopathology nor rejected by an uneventful 1-year follow-up regarding infectious disease without further antibiotic treatment.

Image acquisition and analysis

Depending on the centre, images had originally been acquired on a Biograph mCT (Siemens Healthcare, Erlangen, Germany) or a Gemini TF PET/CT (Philips Medical Systems, Eindhoven, The Netherlands).

Image quality

All scans were evaluated for image quality (sharpness/noise) and classified as "good", "moderate" or "poor" by two independent observers. Scans that were classified as being of poor quality were excluded from further analyses. Furthermore, the quality of myocardial FDG uptake suppression was assessed by visually comparing the maximum intensity of FDG in the myocardium to the blood pool (of the descending aorta) and the liver, and classified as (I) "less than blood pool", (II) "equal to blood pool", (III) "less than liver but more than blood pool", (IV) "more than liver" and (V) "intense" (i.e. similar to brain).

Qualitative analysis of PVE

All images were analysed on commercially available software (Syngo.via, Siemens, Erlangen, Germany) by two nuclear medicine physicians blinded to the original scan report and any clinical data of the patient. Each observer had several years of experience in reading FDG PET/CT scans

for suspicion of PVE, device infections and infectious diseases in general. Based on both the attenuation-corrected and non-corrected images, both observers assessed the presence of any uptake around the PV and gave a final verdict on the abnormality of this uptake (consistent with infection), taking known normal variations into account.[9] In case of disagreement, a consensus reading was performed.

(Semi-)quantitative analysis of PVE

The maximum SUV (SUV_{max}) around the PV was measured on EARL-accredited, attenuation-corrected reconstructions. A volume of interest was defined automatically as an isocontour of 40% of the maximum measured signal intensity, which included the blood pool within and some of the soft tissue adjacent to the PV (figure 1). When there was a visual impression of unsuppressed myocardial FDG uptake within the automatically generated volume of interest, this area was manually removed from the measurement. The SUV_{max} was divided by the mean SUV of the blood pool in the descending aorta (at the level of the PV) to calculate the target-to-background ratio (SUV_{ratio}). This was measured within a spherical volume of interest with a maximum diameter of the lumen of the aorta (excluding the vessel wall). For all semiquantitative analyses, the average of the measurements of the two observers was used.

In case of multiple PVs, each valve was assessed and scored separately. Because the definition of case (PVE) versus control (no PVE) was set on a patient-level rather than on a valve-level, only the affected PV was included for the analyses of diagnostic performance and thresholds for SUV_{max} and SUV_{ratio}, to prevent erroneously classifying the scan as false-negative for the other valve(s). In patients with multiple valves without PVE (all negative on FDG PET/CT), the most commonly implanted valve was considered the primary valve (i.e. in the following order: aortic, mitral, pulmonary and tricuspid valve). Only the SUV_{max} around the

primary valve was included in the analysis. Scans that were not acquired on an EARL-accredited scanner or did not include an EARL-accredited reconstruction were not included in the semiquantitative analyses.

Confounders

The effect of possible confounders on the diagnostic accuracy was assessed by identification of statistically significant differences between patients with false positive or false negative interpretations and those with correctly interpreted scans using a logistic regression model including all demographic and clinical variables (supplementary table 1). In particular, the effect of the previously described potential positive (i.e. recent valve implantation and surgical adhesives) and negative (i.e. low inflammatory activity due to prolonged antibiotic therapy and isolated vegetations) confounders was evaluated. The effect of poor myocardial FDG-uptake suppression was evaluated both as a possible predictor of false positive (i.e. uptake that mimics an infectious pattern) and false negative (i.e. diffuse myocardial uptake that masks an underlying infectious pattern) scans. Subsequently, all patients or scans with statistically significant predictors of a negative or positive confounding effect were excluded to evaluate possible improvement of diagnostic accuracy.

Statistics

Accuracy of FDG PET/CT for the diagnosis of PVE was assessed by comparing the final imaging diagnosis (based on visual analysis) to the reference standard of expert consensus based on all available clinical data. For comparisons between groups, the Student's *t*-test or Mann-Whitney U-test were used for normally distributed and non-parametric data, respectively. For all statistical analyses, a significance level of $\alpha=0.05$ and 95% confidence intervals (CI) were used. SPSS v25.0 (IBM Corp., Armonk, NY) was used for all analyses except confidence intervals for

C-statistics, for which MedCalc v18.2.1 (MedCalc Software, Seoul, Republic of Korea) was used. Unless otherwise indicated, the inter-quartile range (IQR) or CI are denoted in square brackets.

The inter-observer agreement on quantitative analyses was evaluated using an absolute-agreement two-way-mixed intra-class correlation coefficient.[18] Receiver-operator curves (ROC) were used to analyse diagnostic distinctiveness (as area under the curve, AUC) and the optimal cut-off values for both SUV_{max} and SUV_{ratio} were determined using Youden's J statistic, assuming that sensitivity and specificity are equally important. Outcomes of the quantitative analyses were adjusted for the same confounders identified in the visual assessment through exclusion of the same scans.

Results

Patient characteristics

Between January 2010 and March 2016, 390 FDG PET/CT scans had been acquired in 289 patients with at least one prosthetic heart valve. After exclusion of scans that were irretrievable, had a poor image quality, or for which sufficient clinical data were lacking, and after exclusion of all follow-up scans, 237 FDG PET/CT scans remained (figure 2).

One hundred and sixty scans were performed for suspicion of PVE, while 77 scans were acquired for other, mostly oncological indications. Clinical parameters including blood cultures and results of echocardiography at the time of these scans are listed in table 1. Of the 160 patients suspected of PVE, 80 had a final diagnosis of PVE, while the other 80 were deemed not to have PVE.

Acquisition parameters and image quality

Acquisition parameters of the 237 FDG PET/CT scans are shown in supplementary table 2. Overall image quality was good in 161 and moderate in 76 scans. All patients had fasted for at least 6 hours, while 88 of the patients suspected of PVE (55%) had also been on a low-carbohydrate diet for at least 24 hours prior to FDG injection, of whom 20 (12%) had additionally received a heparin injection (50 IU/kg) 15 minutes before FDG injection. One patient in the negative control group who underwent FDG PET/CT for suspicion of cardiac sarcoidosis had also been on a low-carbohydrate diet for more than 24 hours.

The preparatory low-carbohydrate diet (n=88) significantly reduced the average grade of physiological myocardial FDG uptake from 3.0 to 2.0 on a 1-5 scale when compared to the patients who were not instructed to adhere to this diet for 24 hours ($p<0.001$, supplementary table 3). An additional intravenous heparin injection (n=20) reduced the average grade of myocardial FDG uptake further, from 2.0 to 1.6 ($p=0.02$). Both methods individually led to a significantly lower percentage of scans in which the observers indicated that myocardial uptake may have negatively influenced their quantitative measurements of FDG uptake, and combined, they reduced the amount of possibly affected measurements from 31% to 12% ($p<0.001$, supplementary table 3).

Visual assessment

Visual assessment of FDG uptake by two independent blinded observers resulted in 66 positive and 171 negative PET/CT scans. After a consensus reading of 23 scans (10%), there were no remaining discrepancies between the two observers in the evaluation of pathological FDG uptake.

In the 160 PET/CT scans performed for suspicion of PVE, PET was positive in 59 out of the 80 patients with PVE, and negative in 74 out of 80 patients without PVE. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of scans acquired for suspicion of PVE, without any correction, were 74%, 91%, 89% and 78%, respectively. When only looking at patients with a surgically and/or histopathologically confirmed diagnosis, PET was positive in 32 out of 40 patients (80%).

The sensitivity of echocardiography for PVE was 50/77 (65%, see table 1). In 24 out of the 27 cases of PVE in which echocardiography was negative, the FDG PET/CT scan was positive. Thus, when adding FDG PET/CT to the diagnostic work-up, the combined sensitivity increased to 96%. Of the 53 patients deemed not to have PVE of whom an echocardiogram report was available, echocardiography showed signs of endocarditis in 8 (specificity 85%). None of them had a positive FDG PET/CT scan (combined specificity 100%).

False negative interpretations

Overall, 21 scans of patients with PVE (26%) were false negative based on the visual analysis. Fourteen of these patients had a definite diagnosis of PVE according to the modified Duke criteria, while 7 had a possible PVE classification.

As a measure of inflammatory activity, the average CRP at time of PET imaging was lower in these 21 patients than in those with a true positive scan (25.0 ±24.5 vs. 73.7 ±59.9 mg/L; p=0.001). At the time of these 21 scans, 17 patients (81%) had a CRP of less than 40mg/L (4x upper normal limit), which was a statistically significant predictor of a false-negative FDG PET/CT scan (p=0.016, figure 3A). When excluding all 69 scans of patients with a CRP of less than 40mg/L at the time of imaging (n=91), the sensitivity, specificity, PPV and NPV of visual PET analysis for PVE (in patients with a CRP of more than 40mg/L) improved to 91%, 91%,

91% and 91%, respectively. None of the other variables (including leukocyte levels at the time of imaging) were significant predictors of a false negative scan, although longer intravenous antibiotic treatment was associated with lower inflammatory activity (Spearman correlation coefficient of -0.36 mg/L CRP for every day of antibiotic therapy, $p<0.001$). Specific species of micro-organisms were not associated with lower inflammatory activity at the time of PET/CT imaging, nor with a false-negative FDG PET/CT scan.

Of the four patients with a false-negative visual interpretation of their FDG PET/CT scan despite higher CRP levels, three had an isolated vegetation on their aortic PV without any signs of structural damage to the peri-annular tissue. The fourth patient with a false-negative FDG PET/CT had negative blood cultures, but was deemed to have PVE by expert consensus due to echocardiographic signs of a peri-annular extension, for which the patient was however not reoperated due to a too-high estimated procedural risk. Thus, absolute certainty about the diagnosis of PVE in these patients could not be achieved, but they were all pragmatically antibiotically treated for at least 6 weeks despite a negative FDG PET/CT scan.

False positive interpretations

Six scans in patients suspected of PVE but with a final rejected diagnosis (8%) were false positive.

In three of these patients, the surgical report of the PV implantation mentioned use of surgical adhesives (i.e. BioGlue [CryoLife Inc., Kennesaw, GA, USA]), which was the only statistically significant predictor of a false positive scan in the logistic regression model. The area of increased FDG uptake was consistent with the description of the area the surgical adhesive had been applied to in the surgical report of all three patients (figure 3B). There were no negative scans in patients in whom use of surgical adhesives had been reported (n=4).

Excluding scans affected by this confounder increased the specificity and PPV of PET in patients suspected of PVE to 96% (74/77) and 95% (58/61), respectively.

In two other patients, the PV had been implanted 11 and 18 days prior to FDG PET/CT imaging, respectively. However, 16 other patients who underwent imaging within one month of PV implantation had a true negative FDG PET/CT scan. Overall, in 20 patients the PV had been implanted less than 1 month before FDG PET/CT imaging, with only two false positive FDG PET/CT scans (10%). Two other patients with an implantation this shortly before FDG PET/CT imaging were deemed to have PVE: one scan was true positive, the other false negative. Twenty-seven scans had been acquired in patients with a PV implantation between one and three months before imaging, with only two false positive FDG PET/CT scans in this group as well (7%). One of these false positives within 1-3 months after surgery was also attributed to the use of BioGlue, bringing the total number of false positive scans in patients with a PV implantation less than three months before FDG PET/CT imaging to 3/47 (6%). Neither the number of days since PV implantation, nor an implantation within one or three months, were significant predictors of a false-positive scan.

In the last patient suspected of PVE who had a false positive scan, as well as in the one patient with a false positive scan that had been acquired for oncological indications, myocardial FDG uptake had been classified by both observers as ‘more than liver’ and ‘intense’, respectively, which may have hampered the visual assessment (figure 3C). Neither patient had been prepared by means of a low-carbohydrate diet. However, since poor myocardial suppression occurred in many more correctly evaluated FDG PET/CT scans, this was not a statistically significant confounder either.

Diagnostic performance adjusted for significant confounders

Following the exclusion of scans influenced by low inflammatory activity at time of imaging and those influenced by prior use of surgical adhesives, the sensitivity, specificity, PPV and NPV of FDG PET/CT in patients suspected of PVE increased to 91%, 95%, 95% and 91%, respectively. Recent valve implantation (within one or within three months before FDG PET/CT imaging) was not a significant confounder.

Quantitative analysis

Of the 237 included scans, 170 had an EARL-accredited reconstruction available for (semi-) quantitative analyses: 55 in the PVE group and 115 in the control groups. The mean SUV_{max} and SUV_{ratio} (average of both observers) in the PVE group were significantly higher than in both the group of patients suspected of PVE with a final rejected diagnosis and the negative control group (table 2, figure 4).

A total of 52 EARL-standardized scans (10 in the PVE group, 14 in the no-PVE after suspicion group, and 28 in the negative control group) were affected by unsuppressed myocardial FDG uptake in such a way that one or both observers had indicated that this may have influenced their measurements. However, excluding these scans from the analyses did not substantially change the average values in any of the groups. When excluding scans that may have been confounded by either low inflammatory activity (CRP < 40mg/L) or prior use of surgical adhesives on the other hand, the differences between the PVE group and the no-PVE groups increased (table 2, figure 4). In particular, two scans with very high values due to surgical adhesives, were removed from the control group by this correction. There were no significant differences in SUV_{max} or SUV_{ratio} between the patients initially suspected of PVE with a rejected diagnosis and those who underwent FDG PET/CT imaging for other indications.

Only looking at EARL-standardized scans obtained for suspicion of PVE (n=111) and assuming sensitivity and specificity are equally important, the optimal threshold was 4.2 for SUV_{max} (sensitivity 60%, specificity 91%, PPV 87%, NPV 70%, AUC 0.81 [0.73–0.88]) and 2.1 for SUV_{ratio} (sensitivity 75%, specificity 86%, PPV 84%, NPV 77%, AUC 0.83 [0.75–0.89]; figure 5A).

When excluding scans in patients with either a CRP of less than 40mg/L or prior use of surgical adhesives (n=64 remaining), the diagnostic performance of both cut-off values improved substantially, with an optimal cut-off value of 3.3 for SUV_{max} (sensitivity 97%, specificity 79%, PPV 81%, NPV 96%, AUC 0.95 [0.87–0.99]) and 2.0 for SUV_{ratio} (sensitivity 100%, specificity 91%, PPV 91%, NPV 100%, AUC 0.99 [0.93-1.00]; figure 5B). Adjusted for confounders, an SUV_{ratio} of 2.6 had a specificity (and PPV) of 100% for PVE (figure 5B).

When verifying these cut-off values in the negative control group without any suspicion of PVE, 4/59 scans had an SUV_{ratio} of more than 2.0 (specificity 93%) while none were higher than 2.6. Three of these four measurements had been done in oncological scans with intense myocardial uptake due to a lack of adequate patient preparation, which may have influenced these measurements. The fourth had an SUVratio of 2.1.

Interobserver variability

Overall, in the EARL-standardized scans (n=170), the differences between SUV_{max} and SUV_{ratio} measurements of both observers were relatively small (mean SUV_{max} difference -0.04, 95% CI -0.35–0.28, p=0.82; mean SUV_{ratio} difference 0.04, 95% CI -0.16–0.25, p=0.70). The two-way mixed intra-class correlation coefficient of absolute agreement for single SUV_{max} measurements (0.82, 95% CI 0.76-0.86) and SUV_{ratio} measurements (0.84, 95% CI 0.78-0.88) indicated a good-to-excellent agreement between the observers on both variables. Excluding scans with

unsuppressed myocardial FDG uptake that may have hampered these measurements increased interobserver reliability to excellent for both variables, with intra-class correlation coefficients of 0.94 (95% CI 0.91–0.96) and 0.95 (95% CI 0.92–0.96) for SUV_{max} and SUV_{ratio}, respectively.

Discussion

To our knowledge, this multicentre study reports the largest patient cohort on the diagnostic performance of FDG PET/CT in PVE to date, including a negative control group of patients with a PV who underwent FDG PET/CT imaging for other indications than suspected PVE. While several authors have reported the possible influence of prolonged antibiotic therapy and surgical adhesives, to our knowledge this study is the first to identify these factors as significant confounders and to assess the true diagnostic accuracy of FDG PET/CT by excluding scans that were affected by them. Finally, in this study, standardized quantification of FDG uptake after exclusion of these confounders allowed for identification of a reliable diagnostic cut-off for PVE that can be used in any centre with an EARL-calibrated scanner (currently over 150 centres in Europe[16]).

The diagnostic performance of the visual assessment of FDG PET/CT scans in patients suspected of PVE in our study, not adjusted for confounders, was reasonable and comparable to previous studies[12,19], with a sensitivity, specificity, PPV and NPV of 74%, 91%, 89% and 78%, respectively. As previously mentioned, low inflammatory activity at time of FDG PET/CT imaging and prior use of surgical adhesives during PV implantation were respectively identified as significant predictors of false negative or false positive misinterpretations in a logistic regression model. Excluding scans affected by these two significant confounders significantly

improved the diagnostic performance values of the visual assessment in patients suspected of PVE to 91%, 95%, 95% and 91%.

Confounding factors

Low inflammatory activity

Several authors of previous studies regarding FDG PET/CT in suspected PVE have suggested the influence of low inflammatory activity −measured by CRP or white blood cell count− or prolonged antibiotic therapy on false negative PET interpretations.[8,19] However, no studies to date had corrected for this confounder, even though several studies on the value of FDG PET/CT for the detection of infections of unknown origin have shown that high inflammatory activity is a significant predictor of −and may be a requirement for− an adequate FDG PET/CT scan.[20,21]

The drastic increase in sensitivity observed when adding FDG PET/CT to echocardiography (from 65% of echocardiography alone to 96% of both imaging techniques combined), without even adjusting for low inflammatory activity, is probably caused by the fact that the patients with negative echocardiograms are often also the patients that were scanned early in the disease process, before structural damage or vegetations ensued. It is in these patients that levels of inflammatory activity are still high and that FDG PET/CT is most reliable.

Whether initial or empirical antibiotic therapy should be ceased for diagnostic purposes, however, −in case inflammatory parameters have already diminished without a certain diagnosis− remains questionable in light of the risks associated with unsuccessfully treated PVE. In our opinion, a pragmatic antibiotic treatment of a 'possible PVE' is preferable over a PET-confirmed definite PVE that has to be reoperated due to cessation of antibiotic therapy. While a CRP level of less than four times the upper normal limit (<40mg/L) was a significant and major predictor of false-negative interpretations in this study, FDG PET/CT may still be considered

when inflammatory parameters are low if the diagnosis of PVE has significant therapeutic consequences. In our study, FDG PET/CT was positive in 13/28 (46%) patients with a CRP of <40mg/L and a definite diagnosis of PVE.

Surgical adhesives

Surgical adhesives are known to be very FDG-avid, with several case reports on patients who underwent lung surgery, aortic surgery or heart valve surgery showing intense FDG uptake in areas where they had been applied, which can persist for several years if not indefinitely.[9] In our study, we evaluated the surgical reports of all patients, of which four mentioned the use of a surgical adhesive during PV implantation. All four of these patients (three without PVE, one with PVE) had a positive FDG PET/CT scan, and as far as could be determined from the surgical report, the areas of FDG uptake were consistent with the areas that these adhesives had been applied to.

Recent valve implantation

Current ESC guidelines recommend not to perform FDG PET/CT within three months of PV implantation.[4] The reasoning behind this three-month grace period was the assumed likelihood of false-positive findings due to sterile inflammation –as seen in recent lung cancer resection surgery[22]– based on expert opinion and a case report of increased FDG uptake around a biological mitral PV implanted two months prior to FDG PET/CT imaging. In the largest study on FDG PET/CT in suspected PVE prior to the 2015 update of the ESC guidelines, Saby *et al.* excluded patients with a PV implanted less than one month before admission to avoid false-positive results related to early post-operative inflammation. They also referred to the same case report, but described no false positives that could have been attributed to PV implantation between two and three months prior to imaging. In another study by Rouzet *et al.*, in which FDG

PET/CT was compared with radiolabelled leukocyte scintigraphy in patients suspected of PVE, six patients in whom a PV had been implanted less than two months before imaging had a false positive FDG PET/CT result, while leukocyte scintigraphy was not affected by this sterile inflammation.[23] Since the publication of this study and the 2015 update of the ESC guidelines, however, several studies that included patients scanned within three months of implantation, some even within two weeks, have explicitly described true negative findings.[11] Moreover, Mathieu *et al.* recently described a cohort of 51 patients without PVE, and showed that the mean amount of FDG uptake was not significantly different between patients scanned within three months of implantation or thereafter, and that elevated FDG uptake may occur as late as eight years after PV implantation without any clinical suspicion of PVE.[24] In our study, recent valve implantation was not a significant predictor of false positive interpretations, and we cannot substantiate the ESC guideline recommendation. We believe performing FDG PET/CT early after surgery poses no significant diagnostic difficulties based on our findings and the evidence available from previous studies.

Myocardial FDG uptake

Visual assessment of FDG uptake around the PV was not significantly hampered by unsuppressed myocardial uptake in this study, even though the myocardial uptake had been classified as 'more than liver' or 'intense' in a substantial number of scans. Particularly in patients who had not been prepared with a low-carbohydrate diet prior to the scan, myocardial FDG uptake was frequently intense. Unsuppressed myocardial FDG uptake did not hamper the quantitative measurements as much as we had initially anticipated, either, as demonstrated by the merely slight change in average SUV_{max} and SUV_{ratio} when excluding scans with myocardial uptake that may have possibly affected the observers' measurements. However, it did

significantly decrease inter-observer reliability, and should always be minimized as much as possible using at least a >6-h fast (in our own experience, preferably at least 12-h) and a 24-h low-carbohydrate diet to allow for easy distinction of periprosthetic FDG uptake.[10,15] An additional i.v. injection of 50 IU/kg of unfractionated heparin 15 minutes before FDG administration (on top of the prolonged fasting and low-carbohydrate diet) slightly further reduced myocardial FDG-uptake (supplementary table 3), but the sample size was too small to show a clinically meaningful difference and the small potential additional benefit of this should always be weighed against the possible adverse effects.[25–27] Without a low-carbohydrate diet or extensive fasting (>18h) however, the additional value of i.v. heparin seems limited and does not result in sufficient myocardial suppression.[28] Furthermore, patients already on low molecular weight heparin or warfarin therapy most likely already benefit from the incremental suppression these drugs provide, and do not need an additional unfractionated heparin bolus injection.[29]

Quantitative analysis

Quantification of FDG uptake, expressed as SUV_{max} or SUV_{ratio}, showed reasonable diagnostic performance with an AUC for SUV_{max} of 0.81 and an AUC for SUV_{ratio} of 0.83, but lacked sufficient sensitivity. However, after exclusion of the previously mentioned significant confounders, the diagnostic performance drastically increased and more reliable cut-offs could be identified.

Pizzi *et al.* reported an AUC for SUV_{max} and SUV_{ratio} of 0.89 in a prospective study comprising 92 patients suspected of PVE, with a sensitivity/specificity for their cut-offs ($SUV_{max}\geq3.7$, $SUV_{ratio}\geq1.69$) of 91%/79% and 91%/76%, respectively.[12] Their measurements had not been performed on EARL-standardized reconstructions. After exclusion of scans affected by confounders, we found slightly different cut-offs for both measures ($SUV_{max}\geq3.3$,

SUV_{ratio}≥2.0) with a sensitivity/specificity of 97%/79% and 100%/91% respectively. SUV_{ratio} was the most reliable and predictive measure, possibly due to it being less dependent on patient characteristics and scanning parameters, with a 91% PPV for a SUV_{ratio} of ≥2.0 and a 100% PPV for a SUV_{ratio} of ≥2.6.

As a confirmation of validity, there were no significant differences in measured SUV_{max} or SUV_{ratio} between the group of patients initially suspected of PVE with a final rejected diagnosis and the negative control group of patients who underwent FDG PET/CT imaging for other indications.

Some authors have suggested to calculate the SUV_{ratio} by dividing by the mean SUV in the atrial or mediastinal blood pool (as opposed to the blood pool in the descending aorta) because some patients may show increased FDG uptake in the aortic wall due to aortic calcifications or active plaque.[13,14] In our study, we took particular care not to include the vessel wall in the volume of interest in the descending aorta, and preferred to adhere to the most commonly used measurement method for comparison.[10] Additionally, in case of valvular regurgitation or atrial fibrillation, the atrial wall may similarly show increased FDG uptake.

Inter-observer reliability

Inter-observer agreement for quantitative measurements of FDG uptake was good-to-excellent in our study, and significantly improved when excluding scans with poorly suppressed myocardial FDG uptake. This shows that quantification of perivalvular FDG uptake is a reliable tool with diagnostic cut-off values applicable to all centres with EARL accreditation, which allows for less-subjective image evaluation. There were, however, some discrepancies between the observers in the initial visual assessment of a number of scans (10%), for which a consensus reading was performed. In the majority of these, experience with normal variations of

perivalvular uptake, which one observer arguably had less than the other, was key to a correct interpretation, exemplifying that the visual assessment of paravalvular FDG uptake is not always black and white and probably subject to a learning curve. While the exact role of the pattern and distribution of FDG uptake around a PV (e.g. heterogeneous, diffuse or focal) is still unclear, some patterns such as diffuse slightly-increased FDG uptake have been attributed to physiological inflammation processes around the PV.[24]

Limitations

Our study had a number of potential limitations. Most importantly, besides the regular limitations of a retrospective study design, our study may have been influenced by the availability of the FDG PET/CT results to the expert team determining the final diagnosis, which may have introduced an incorporation bias. This is, however, the case in all studies to date regarding novel imaging techniques for suspected PVE, and is hard to circumvent. Even when investigators are blinded to the FDG PET/CT results, the subsequent clinical course of action will usually reveal the implications that the FDG PET/CT findings had. Ideally –although imaginably difficult and possibly unethical to realize–, the diagnostic performance of FDG PET/CT would be evaluated in a prospective trial in which even the physicians remain blinded to its findings. Additionally, while we strongly believe FDG PET/CT may aid in the timely diagnosis of PVE, the impact of an early diagnosis of PVE by FDG PET/CT on morbidity and mortality remains uninvestigated, and would require large, randomized-controlled trials to be elucidated.

Secondly, all FDG PET/CT scans were reassessed by two independent observers with several years of experience in FDG PET/CT imaging of suspected PVE, who were blinded to all clinical data. The external validity of a fully blinded assessment of FDG PET/CT in suspected

PVE could be contested, as clinical information is often important for the interpretation of possibly pathological FDG uptake. In clinical practice, results of the FDG PET/CT scan would be interpreted in a multidisciplinary setting in the context of the clinical presentation, microbiological and echocardiographic findings as well as results of other imaging techniques such as CT angiography. Furthermore, the blinding may have been imperfect in the presence of obvious findings such as large malignancies, biasing the interpretation by revealing an alternate diagnosis, especially since the observers were aware of our study design. This bias did, however, not have any effect on the calculation of diagnostic accuracy in our study, as oncological scans were not included in these analyses.

Finally, the exclusion of scans affected by significant confounders may have limited clinical applicability and generalizability of our findings. However, the confounders identified in this study can most likely be identified and mitigated in clinical practice as well, most importantly by implementing FDG PET/CT early in the diagnostic work-up of PVE to prevent imaging after extended periods of antibiotic therapy, while insight in the surgical report may help to identify increased FDG uptake due to use of surgical adhesives.

Clinical implications

Our findings may have several important clinical implications. First, our study shows that FDG PET/CT should preferably be implemented early in the diagnostic work-up of suspected PVE to prevent the negative confounding effect of low inflammatory activity, ideally while CRP levels are above 40mg/L (figure 6). Moreover, if implemented early, FDG PET/CT can detect PVE before structural damage occurs, allowing timely appropriate antibiotic treatment which could possibly prevent a reoperation, while also preventing missed diagnoses because echocardiography may be negative in these early stages of the disease.[10] In any patient with a PV

and positive blood cultures, particularly if the micro-organism is known to be aggressive (e.g. *Staphylococcus aureus*)[30], FDG PET/CT should readily be considered in the absence of clear alternative diagnoses. Second, if performed timely and taking into account possible confounders, EARL-standardized quantification of FDG uptake around PVs (as SUV_{ratio}) has a very high predictive value for PVE at a cut-off of ≥2.0 (100% sensitivity, 91% specificity), which can immediately be applied in daily clinical practice in all EARL-accredited centres. Third, the interpreting nuclear physician has to be explicitly made aware of prior use of surgical adhesives during PV implantation. Fourth, although myocardial FDG uptake did not substantially influence our results, adequate suppression by at least a low-carbohydrate diet is essential for reliable PV assessment, and easy to achieve. And finally, our results do not corroborate ESC guideline recommendations to avoid FDG PET/CT in patients with a recently-implanted PV. The possibility of periprosthetic FDG uptake due to physiological inflammation should always be taken into account, although future studies on the distribution and patterns of FDG uptake may identify characteristics that further aid the distinction between inflammation and infection. Most importantly, however, the goal should never be to replace routine PVE diagnostics (e.g. echocardiography, blood cultures, CTA), but rather to combine all these modalities, each with their specific strengths and weaknesses, in order to achieve optimal diagnostic accuracy (figure 6).

Conclusion

Both visual and quantitative assessment of FDG PET/CT have a high diagnostic accuracy in patients suspected of PVE when implemented early in the diagnostic work-up to prevent the negative confounding effect of low inflammatory activity (e.g. due to prolonged antibiotic therapy). As a quantitative measure of FDG uptake, an EARL-standardized SUV_{ratio} of ≥2.0 is a

100% sensitive and 91% specific predictor of PVE. Recent valve implantation did not significantly influence the diagnostic performance of FDG PET/CT in our study, but surgical adhesives used during implantation did.

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Disclosures

None.

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Table 1. Clinical data at time of FDG PET/CT imaging.

	Suspicion of PVE (n=160)			**No PVE (scan for other indication)**
	All scans for suspicion of PVE	**PVE***	**No PVE (after initial suspicion)***	
Demographics	**n=160**	**n=80**	**n=80**	**n=77**
Age (median [IQR], years)	62 [43-73]	52 [35-68][2]	68 [53-75][1]	73 [65-77]
Gender (male)	108 (68%)	49 (61%)	59 (74%)	55 (71%)
BMI (mean ±SD, kg/m^2)	25.2 ±5.0	24.9 ±5.4	25.6 ±4.6	26.3 ±4.7
Diabetes	23 (14%)	10 (13%)	13 (16%)	18 (23%)
Prior history of endocarditis	33 (21%)[2]	15 (19%)[2]	18 (23%)[2]	5 (6%)[1]
Mortality during follow-up	27 (17%)[2]	10 (13%)[2]	17 (21%)[2]	28 (36%)[1]
Primary valve location**				
Aortic	132 (83%)	61 (75%)	71 (90%)	70 (91%)
Mitral	12 (8%)	6 (7%)	6 (8%)	6 (8%)
Pulmonary	14 (9%)	13 (16%)	1 (1%)[1]	0 (0%)[1]
Tricuspid	2 (1%)	1 (1%)	1 (1%)	1 (1%)
Primary valve type**				
Mechanical	82 (51%)	43 (54%)[2]	39 (49%)[1,2]	26 (34%)[1]
Biological	62 (39%)	34 (43%)	28 (35%)	45 (58%)
TAVI	9 (6%)	1 (1%)	8 (10%)[1]	6 (8%)
Homograft	4 (3%)	2 (3%)	2 (3%)	0 (0%)
Autologous	3 (2%)	0 (0%)	3 (4%)	0 (0%)
Including replacement of ascending aorta (i.e. Bentall)	**21 (13%)**	**15 (19%)**[2]	**6 (8%)**	**2 (3%)**[1]
Time since valve implantation (median [IQR], days)	**685 [82-2216]**	**939 [370-2282]**	**226 [39-1923]**[1,2]	**1104 [299-2268]**
Valves implanted <3 month ago	43 (27%)[1,2]	8 (10%)	32 (40%)[1,2]	4 (5%)
Valves implanted <1 month ago	18 (11%)[2]	2 (3%)	16 (20%)[1,2]	2 (3%)
Secondary prosthetic valve	**21 (13%)**	**9 (11%)**	**12 (15%)**	**7 (9%)**
Aortic + Mitral	12 (8%)	3 (4%)	9 (11%)	7 (9%)
Aortic + Pulmonary	9 (6%)	6 (8%)	3 (4%)	0 (0%)
Cardiac implantable electronic device	**17 (11%)**	**8 (10%)**	**9 (11%)**	**5 (6%)**
Echocardiography data available	**130 (81%)**[2]	**77 (96%)**[2]	**53 (66%)**[1,2]	**0 (0%)**[1]
Signs of PVE$^{\Delta}$	58 (44%)	50 (65%)	8 (15%)[1]	n/a
Vegetation$^{\Delta}$	35 (27%)[¥]	32 (42%)[¥]	3 (6%)[1,‡]	n/a
Aortic$^{\Delta}$	24 (18%)	24 (31%)	0 (0%)[1]	n/a
Mitral$^{\Delta}$	6 (5%)	5 (6%)	1 (2%)[‡]	n/a
Pulmonary$^{\Delta}$	6 (5%)	5 (6%)	1 (2%)[‡]	n/a
Tricuspid$^{\Delta}$	2 (2%)	1 (1%)	1 (2%)[‡]	n/a
Abscess$^{\Delta}$	18 (14%)	17 (22%)	1 (2%)[‡]	n/a
Fistula$^{\Delta}$	1 (1%)	1 (1%)	0 (0%)	n/a
Prosthetic valve dehiscence$^{\Delta}$	1 (1%)	1 (1%)	0 (0%)	n/a
Paravalvular leakage$^{\Delta}$	12 (9%)	8 (10%)	4 (8%)[‡]	n/a
Cardiac implantable device infection$^{\Delta}$	3 (2%)	2 (3%)[β]	1 (2%)[β]	n/a

Blood cultures available†	**160 (100%)**[2,§]	**80 (100%)**[2]	**80 (100%)**[2]	**0 (0%)**[1]
Positive blood cultures	86 (54%)	62 (78%)	24 (30%)[1]	n/a
Staphylococcus aureus	28 (18%)	19 (24%)	9 (11%)	n/a
Enterococci	15 (9%)	9 (11%)	6 (8%)	n/a
Coagulase-negative staphylococci	7 (4%)	4 (5%)	3 (4%)	n/a
Viridans streptococci	21 (13%)	17 (21%)	4 (5%)[1]	n/a
HACEK***	5 (3%)	5 (6%)	0 (0%)	n/a
Cutibacterium acnes◊	5 (3%)	5 (6%)	0 (0%)	n/a
Other	5 (3%)	3 (4%)	2 (3%)	n/a
PCR and/or serology positive†,‖	**5 (3%)**	**5 (6%)**	**0 (0%)**	**0 (0%)**[1]
Coxiella burnetii (Q-fever)	2 (1%)	2 (2%)	0 (0%)	n/a
Bartonella henselae	1 (1%)	1 (1%)	0 (0%)	n/a
Tropheryma whipplei	1 (1%)	1 (1%)	0 (0%)	n/a
Haemophilus parainfluenzae	1 (1%)	1 (1%)	0 (0%)	n/a
Modified Duke classification[2]				
PVE rejected	57 (36%)	5 (6%)	52 (65%)[1]	n/a
Possible PVE	45 (28%)	19 (24%)	26 (33%)	n/a
Definite PVE	58 (36%)	56 (70%)	2 (3%)[1]	n/a
Surgical/histopathological confirmation of PVE	40 (25%)	40 (50%)	0 (0%)[1]	n/a
Days of i.v. antibiotic therapy (median [IQR])	11 [6-20]	12 [6-18]	9 [6-23]	n/a
CRP (median [IQR], mg/L)	54 [20-96]	51 [26-70]	63 [17-145]	n/a
Leukocytes (median [IQR], $x10^9$/L)	9.6 [7.3-11.2]	8.6 [6.3-11.0]	9.8 [7.5-11.8]	n/a

1 Statistically significantly different from the PVE group ($p<0.05$, only calculated for No PVE groups). 2 Statistically significantly different from the scans performed for other indications ($p<0.05$, calculated for all scans for suspicion of PVE). * Final diagnosis based on surgical findings (if reoperated), expert opinion and follow-up. ** The primary valve was defined as either the valve involved in -or suspected of- PVE, or in case of controls, based on the order of most common occurrence (AV, MV, PV, TV). ¥ Three patients had a vegetation on both their prosthetic AV and their prosthetic MV. *** HACEK: *Haemophilus* species, *Aggregatibacter* (*Actinobacillus*) species, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella* species. ◊ Formerly known as *Proprionibacterium*. † For blood cultures, PCR and serology, even if they were no longer positive at the time of FDG PET/CT imaging, these were classified as positive if they had been positive during the clinical episode preceding the scan. ‡ The mitral valve vegetation was deemed a remainder of previous endocarditis, while the pulmonary and tricuspid vegetation were later concluded to have probably been small thrombi. The one abscess found in a patient concluded not to have PVE was found to be a remainder of previous endocarditis prior to PHV implantation, as it had decreased in size in comparison with imaging before surgery. Four pre-existing paravalvular leakages were found in this control group. Δ Percentages of abnormal echocardiographic findings are relative to the number of available echocardiograms. β Two patients were deemed to have both PVE and a cardiac implantable electric device infection. One patient was concluded to have a pacemaker lead infection but not PVE, based on absence of abnormalities on echocardiography, CT angiography and PET/CT. ‖ Only PCRs and serologies that newly identified a causative micro-organism are reported. § In two patients, blood cultures were not performed or data about these could not be retrieved from the electronic patient file.

Table 2. Quantification of FDG uptake (averages of both observers, range in square brackets) in all EARL-standardized scans (n=170).

	Suspicion of PVE (n=111)		
	PVE*	**No PVE (after initial suspicion)***	**No PVE (scans for other indications)**
All EARL-standardized scans	**n=55**	**n=56**	**n=59**
SUV_{max} (mean ±SD [range])	4.7 ±1.6 [2.3-9.9]	3.3 ±1.3 [1.3-9.1] (p<0.001)ß	3.4 ±0.7 [2.1-6.7] (p<0.001)ß
SUV_{ratio} (mean ±SD [range])	2.8 ±0.9 [1.4-5.3]	1.9 ±0.8 [0.9-6.8] (p<0.001)ß	1.9 ±0.7 [1.3-6.4] (p<0.001)ß
Scans with sufficient myocardial suppression†	**n=45**	**n=42**	**n=31**
SUV_{max} (mean ±SD [range])	4.8 ±1.6 [2.3-9.9]	3.2 ±1.2 [1.3-9.1] (p<0.001)ß	3.0 ±0.5 [2.1-4.7] (p<0.001)ß
SUV_{ratio} (mean ±SD [range])	2.8 ±0.9 [1.4-5.3]	1.8 ±0.9 [0.9-6.8] (p<0.001)ß	1.6 ±0.2 [1.3-2.1] (p<0.001)ß
Excluding significant confounders‡	**n=30**	**n=34**	**n=10**
SUV_{max} (mean ±SD [range])	5.3 ±1.6 [3.0-9.9]	3.0 ±0.6 [1.8-4.3] (p<0.001)ß	3.6 ±0.7 [2.8-4.9] (p=0.003)ß
SUV_{ratio} (mean ±SD [range])	3.2 ±0.8 [2.0-5.3]	1.7 ±0.3 [1.1-2.6] (p<0.001)ß	1.9 ±0.3 [1.5-2.3] (p<0.001)ß

* Final diagnosis based on surgical findings (if reoperated), expert opinion and follow-up. † Excluding measurements that were indicated as having possibly included unsuppressed myocardial FDG uptake by both observers (n=52, supplementary table 3). ‡ Excluding scans of patients in whom CRP was less than 40mg/L at time of imaging or surgical adhesives had been used during PV implantation. ß Significantly lower than the average measurements in the PVE group.

Figure legends

Figure 1. Semi-quantitative analysis of periprosthetic ^{18}F-FDG uptake in a patient with definite PVE. (A) Horizontal view of a measurement of the SUV_{max} around the PV using an automated volume of interest based on an *isocontour* (red line) encompassing all voxels with an intensity of at least 40% of the voxel with the highest intensity in the spherical selected area (red ellipse). (B) Coronal view of a measurement of the mean SUV in the blood pool of the descending aorta using a small spherical volume of interest (blue circle), drawn with particular care not to include the aortic wall. (C) Combined sagittal view of both measurements. The SUV_{ratio} is calculated by dividing the SUV_{max} around the PV by the mean SUV in the blood pool: 6.97/2.27=3.07.

Figure 2. Inclusion and exclusion flowchart. *EARL*: European Association of Nuclear Medicine Research Ltd.

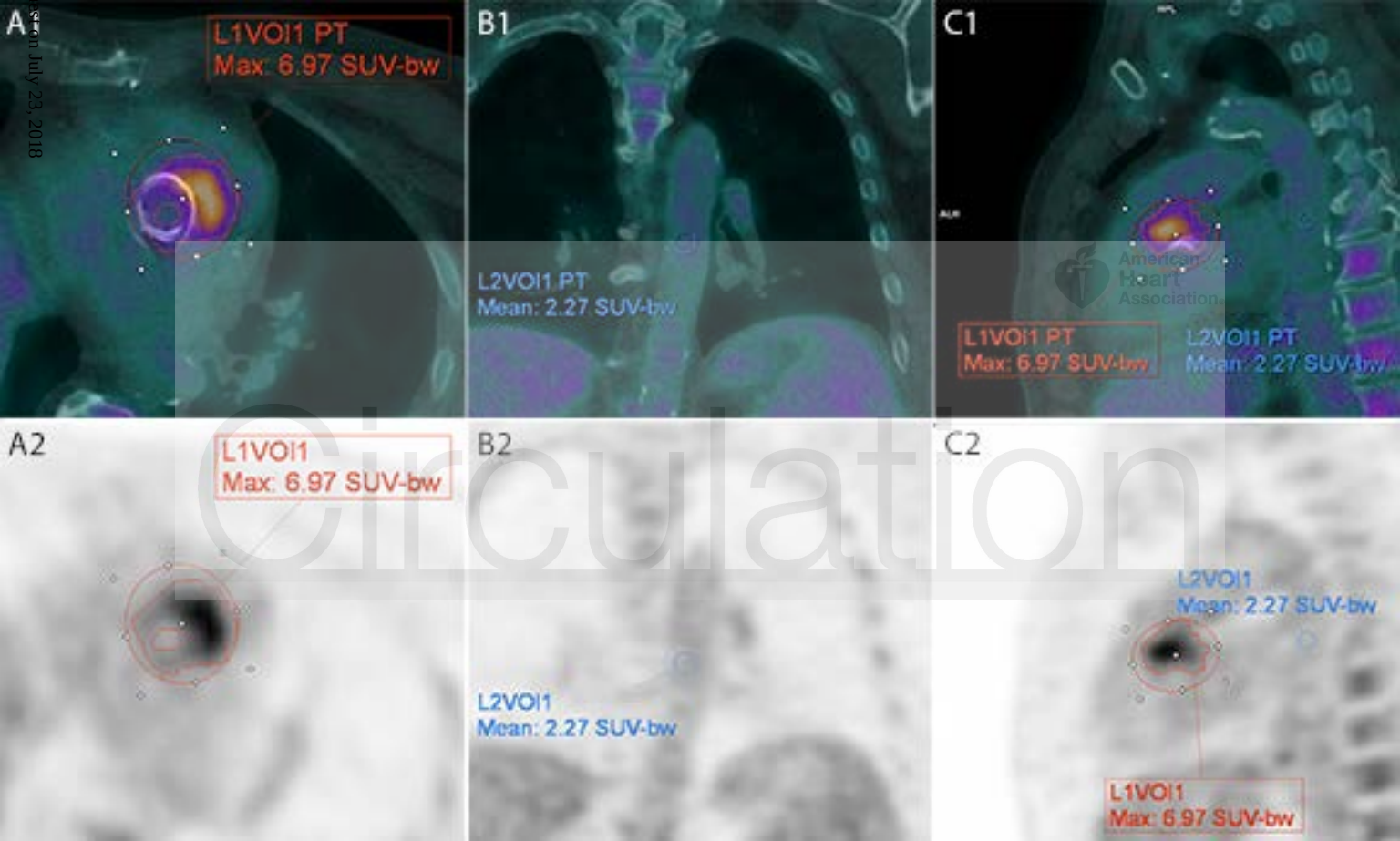
Figure 3. Horizontal (1), coronal (2) and sagittal (3) view of fused FDG PET/CT (1,2) and maximum-intensity PET (3) projections in three patients: (A) an 80-year-old male patient with definite *Enterococcus faecalis* PVE of a biological aortic PV, who had already been treated with antibiotics for 65 days prior to FDG PET/CT imaging (CRP was 14 mg/L), and despite a negative scan (SUV_{ratio} 1.68) was reoperated 5 days later because of persisting vegetations with new septic emboli. PVE was intra-operatively macroscopically and subsequently histopathologically confirmed; (B) a 57-year-old male patient with a biological aortic valve and ascending aorta replacement (Bentall procedure) who underwent FDG PET/CT imaging for oncological indications (myocardial suppression was good, possibly thanks to prolonged >12-h

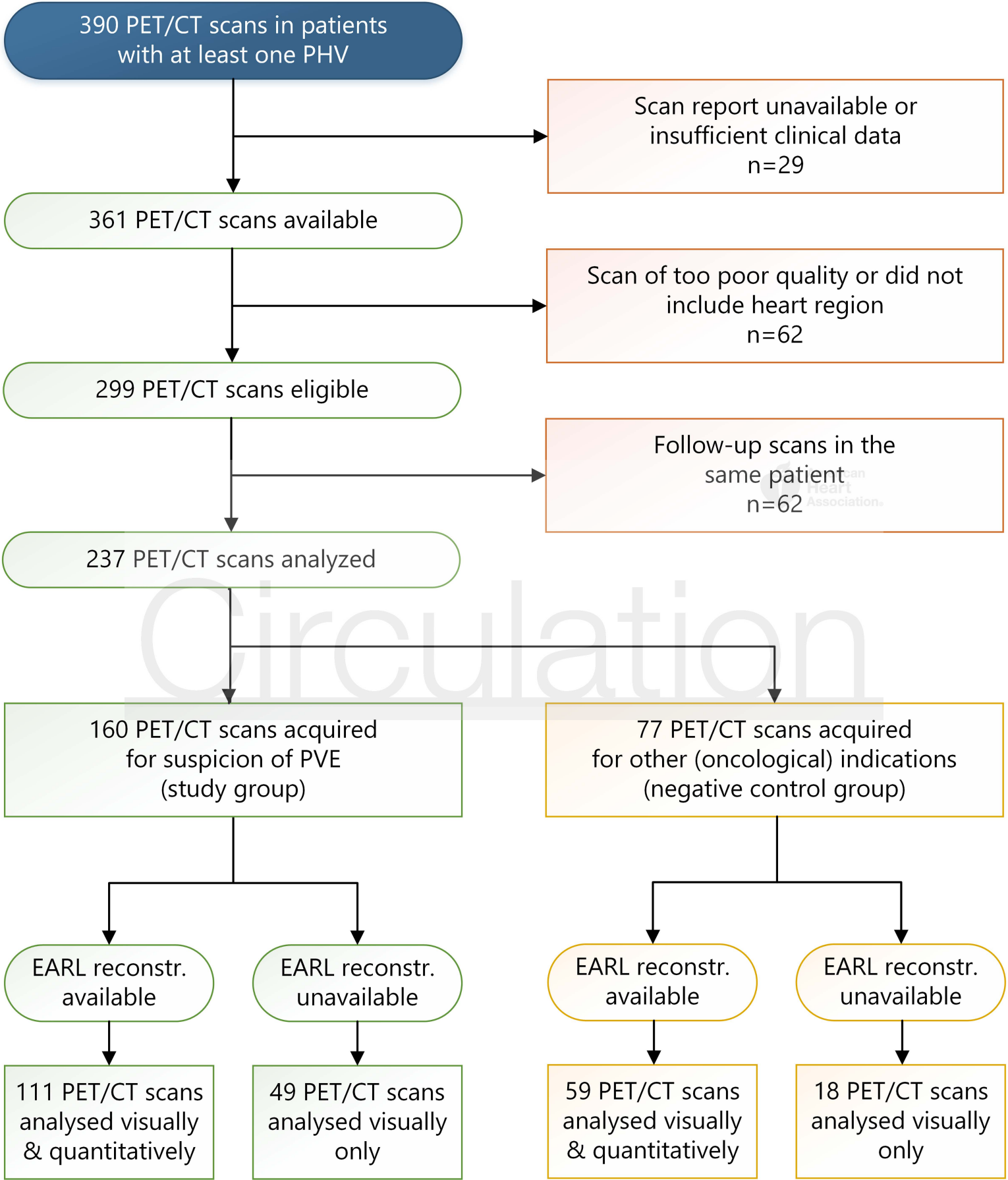
fasting), showing intense uptake of FDG (SUV_{ratio} 6.78) in the areas where surgical adhesives had been applied, particularly surrounding the distal seam of the ascending aortic graft; (C) a 46-year-old male patient with a mechanical aortic valve who underwent FDG PET/CT imaging for suspicion of PVE, but in whom PVE was ruled out by negative blood cultures, negative echocardiography and an alternative diagnosis of upper urinary tract infection, showing circular FDG uptake (C3, SUV_{ratio} 3.66) in the basal septal and anterior myocardial wall (C2) which could have been mistaken for a sign of peri-annular infection (C1), but was most likely caused by insufficient adherence to the low-carbohydrate diet.

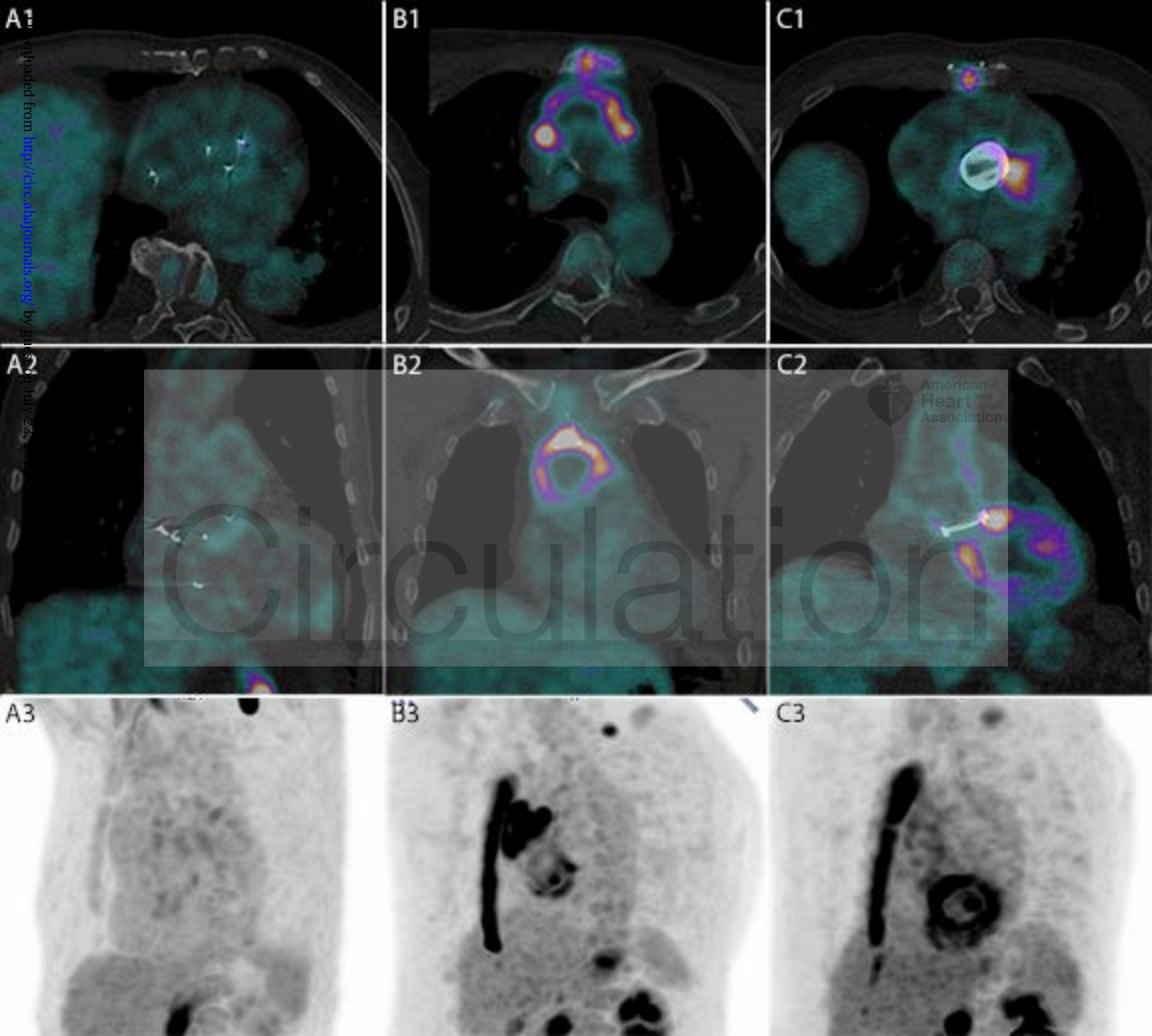
Figure 4. Box-plots for the two-observer average measured SUV_{max} (left) and SUV_{ratio} (right) in cases (red) and controls (green) in all EARL-standardized scans acquired for suspicion of PVE (n=111), and † after exclusion of scans in patients with low inflammatory activity (CRP <40mg/L) at time of imaging or reported use of surgical adhesives during PV implantation (n=64). * Final diagnosis based on surgical findings (if reoperated), expert opinion and follow-up. ‡ Significantly different from adjacent PVE group ($p<0.001$).

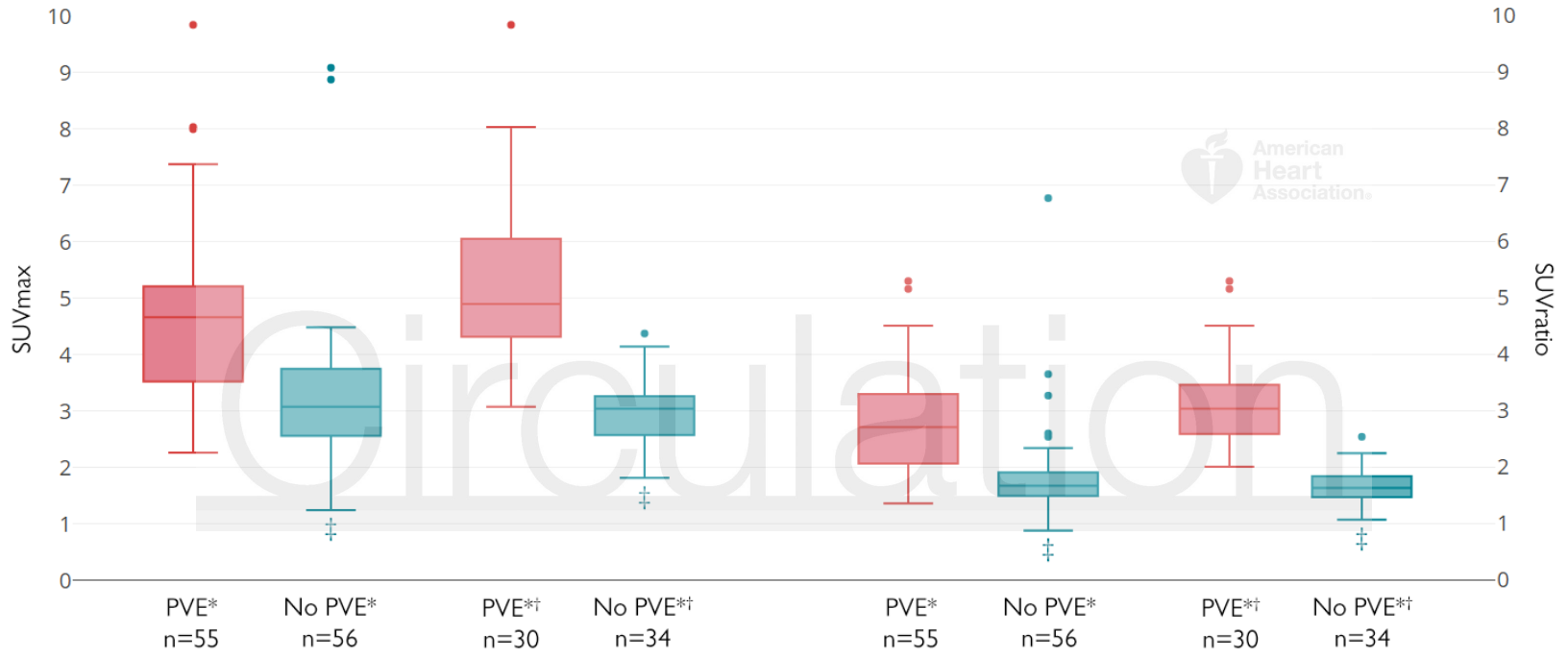
Figure 5. ROC-curves for SUV_{max} and SUV_{ratio} (averages of both observers) in all EARL-standardized scans of patients suspected of PVE (n=111; A) and excluding scans in patients with low inflammatory activity at the time of FDG PET/CT imaging (CRP < 40mg/L) or reported use of surgical adhesives during PV implantation (n=64; B). *Sens*: sensitivity, *spec*: specificity, *AUC*: area under the curve.

Figure 6. Flowchart for the proposed diagnostic work-up of suspected PVE. *TTE*: transthoracic echocardiography; *TOE*: transoesophageal echocardiography; *IE*: international units; *PHV*: prosthetic heart valve; *(^{18}F-)FDG*: 18-Fluorine-Fluorodeoxyglucose; *NAC*: non-attenuation corrected reconstructions; *CT(A)*: computed tomography (angiography); *CAG*: coronary angiography; *CAD*: coronary artery disease.




390 PET/CT scans in patients with at least one PHV
Scan report unavailable or insufficient clinical data n=29
361 PET/CT scans available
Scan of too poor quality or did not include heart region n=62
299 PET/CT scans eligible
Follow-up scans in the same patient n=62
237 PET/CT scans analyzed
160 PET/CT scans acquired for suspicion of PVE (study group)
77 PET/CT scans acquired for other (oncological) indications (negative control group)
EARL reconstr. available
EARL reconstr. unavailable
EARL reconstr. available
EARL reconstr. unavailable
111 PET/CT scans analysed visually & quantitatively
49 PET/CT scans analysed visually only
59 PET/CT scans analysed visually & quantitatively
18 PET/CT scans analysed visually only




SUVmax
SUVratio
10
9
8
7
6
5
4
3
2
1
0
PVE*
n=55
No PVE*
n=56
PVE*†
n=30
No PVE*†
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PVE*
n=55
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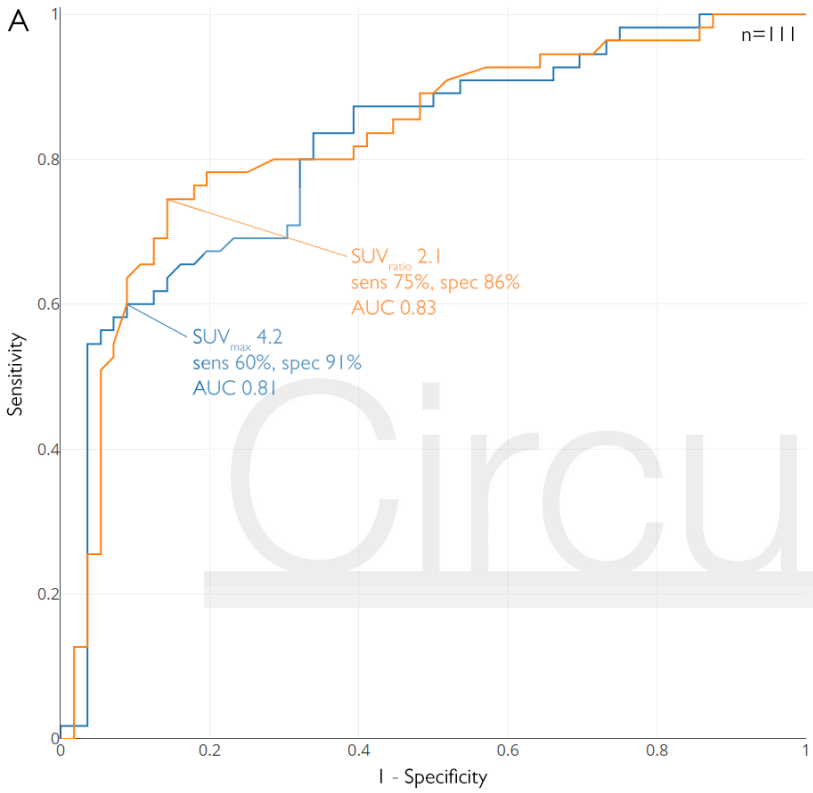
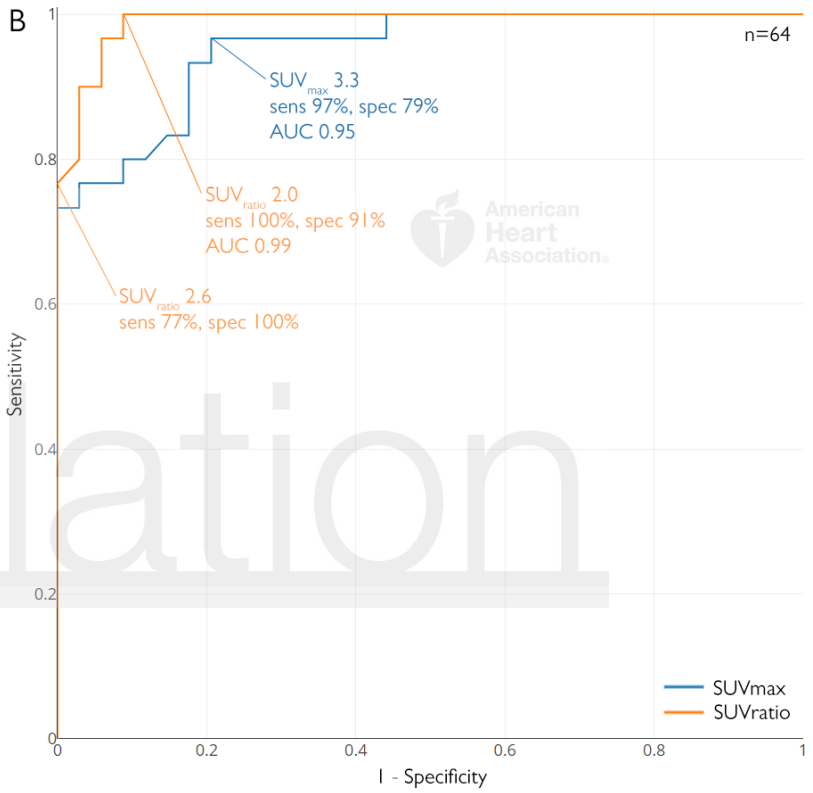
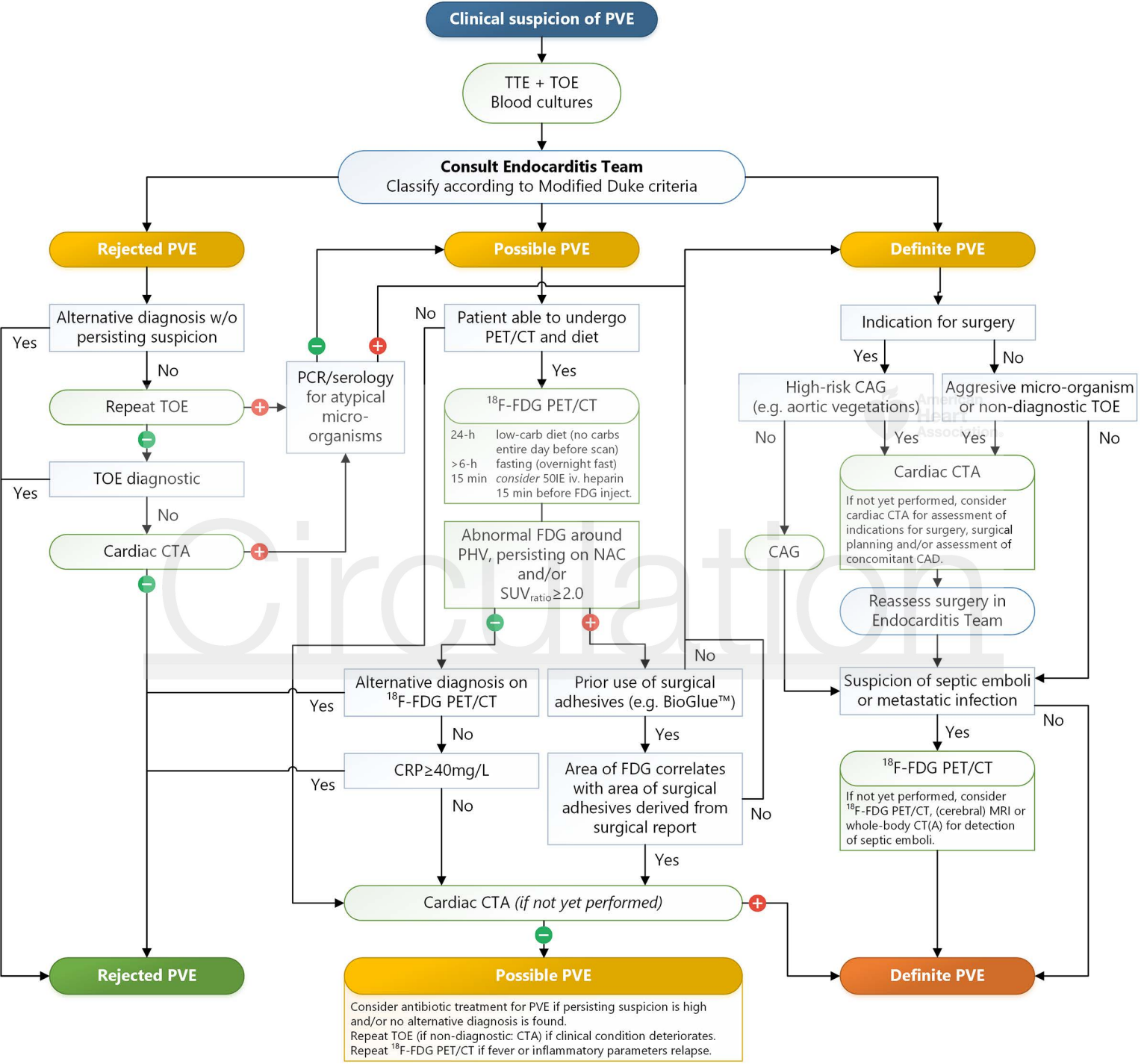
n=56
PVE*†
n=30
No PVE*†
n=34






Clinical suspicion of PVE
TTE + TOE
Blood cultures
Consult Endocarditis Team
Classify according to Modified Duke criteria
Rejected PVE
Possible PVE
Definite PVE
Alternative diagnosis w/o persisting suspicion
Yes
No
Repeat TOE
TOE diagnostic
Yes
No
Cardiac CTA
PCR/serology for atypical micro-organisms
No
Patient able to undergo PET/CT and diet
Yes
18F-FDG PET/CT
24-h low-carb diet (no carbs entire day before scan)
>6-h fasting (overnight fast)
15 min consider 50IE iv. heparin 15 min before FDG inject.
Abnormal FDG around PHV, persisting on NAC and/or SUVratio≥2.0
Alternative diagnosis on 18F-FDG PET/CT
Yes
No
CRP≥40mg/L
Yes
No
Prior use of surgical adhesives (e.g. BioGlue™)
Yes
No
Area of FDG correlates with area of surgical adhesives derived from surgical report
No
Yes
Cardiac CTA (if not yet performed)
Indication for surgery
Yes
No
High-risk CAG (e.g. aortic vegetations)
Aggresive micro-organism or non-diagnostic TOE
No
Yes
Yes
No
CAG
Cardiac CTA
If not yet performed, consider cardiac CTA for assessment of indications for surgery, surgical planning and/or assessment of concomitant CAD.
Reassess surgery in Endocarditis Team
Suspicion of septic emboli or metastatic infection
Yes
No
18F-FDG PET/CT
If not yet performed, consider 18F-FDG PET/CT, (cerebral) MRI or whole-body CT(A) for detection of septic emboli.
Rejected PVE
Possible PVE
Consider antibiotic treatment for PVE if persisting suspicion is high and/or no alternative diagnosis is found.
Repeat TOE (if non-diagnostic: CTA) if clinical condition deteriorates.
Repeat 18F-FDG PET/CT if fever or inflammatory parameters relapse.
Definite PVE

Supplemental Material

Supplementary table 1 – Factors included in the logistic regression model for potential confounders in all scans acquired for suspicion of PVE (n=160).		
	Positive confounding effect	**Negative confounding effect**
Age	n.s.	p=0.09
Gender	n.s.	n.s.
Diabetes	p=0.08	n.s.
Prior history of endocarditis	n.s.	n.s.
Primary valve location	n.s.	n.s.
Primary valve type	n.s.	n.s.
Transcatheter implanted prosthetic valve	n.s.	n.s.
Bentall procedure	n.s.	n.s.
Use of surgical adhesives (e.g. BioGlue)	*p=0.021*	n.s.
Time since implantation	n.s.	n.s.
Secondary prosthetic valve	n.s.	n.s.
Cardiac implantable electronic device	n.s.	n.s.
Signs of PVE on echocardiography	n.s.	n.s.
Positive blood cultures	n.s.	n.s.
Positive PCR and/or serology	n.s.	n.s.
Surgical confirmation of PVE	n.s.	n.s.
Days of i.v. antibiotic therapy*	n.s.	n.s.
CRP	n.s.	*p=0.014*
Leucocytes	n.s.	n.s.

* 21/160 missing data.

Supplementary table 2 – FDG PET/CT acquisition parameters			
	All scans (n=237)	**Scans for suspicion of PVE (n=160)**	**Scans for other indications (n=77)**
Amount of FDG administered (mean ± SD, MBq)	193 ± 69	192 ± 76	198 ± 55
Glucose blood levels at time of injection (mean ± SD, mmol/L)	5.7 ± 1.4	5.3 ± 1.3	6.2 ± 1.5
Time between FDG injection and image acquisition (mean ± SD, minutes)	61 ± 5	61 ± 4	61 ± 6
EARL standardization	170 (72%)	111 (69%)	59 (77%)
At least 6 hours of fasting	237 (100%)	160 (100%)	77 (100%)
Low-carbohydrate diet	88 (37%)	87 (55%)	1 (1%)*
Heparin i.v.	20 (8%)	20 (12%)	0 (0%)

* This scan had been acquired for suspicion of cardiac sarcoidosis, hence the low-carbohydrate diet.

Supplementary table 3 – Influence of patient preparation on myocardial glucose metabolism suppression			
	>6-h fast (n=237)	**>6-h fast +24-h diet (n=88)**	**>6-h fast + 24-h diet + heparin (n=20)**
Mean visual myocardial uptake grading (five-point scale, mean ± SD)	3.0 ± 1.4	2.0 ± 0.8 (p<0.001)*	1.6 ± 0.6 (p=0.02)†
1. Less than blood pool	95 (40%)	57 (64%)	15 (71%)
2. Equal to blood pool	18 (8%)	8 (9%)	2 (14%)
3. More than blood pool, less than liver	15 (6%)	7 (8%)	2 (10%)
4. More than liver	36 (15%)	5 (6%)	0 (0%)
5. Intense	73 (31%)	11 (13%)	1 (5%)‡
Physiological myocardial uptake may have influenced quantitative measurements (in scans with EARL-standardized reconstructions only)	52/170 (31%)	10/57 (18%) (p<0.001)	2/17 (12%) (p=0.03)

* Significant difference when compared to the 149 patients who only fasted for >6 hours and had an average visual myocardial uptake grade of 3.5 ± 1.6. † Significant difference when compared to the 68 patients who fasted and followed a 24-h low-carbohydrate diet but did not get an i.v. heparin injection prior to FDG administration and had an average visual myocardial uptake grade of 2.2 ± 0.8. ‡ The adherence of this patient to the low-carbohydrate was later questioned by the attending physician.