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CXCR4-directed [^{68}Ga]Ga-PentixaFor PET/CT versus adrenal vein sampling performance: a study protocol for a randomised two-step controlled diagnoStic Trial Ultimately comparing hypertenSion outcome in primary aldosteronism (CASTUS)

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ABSTRACT

Introduction Primary aldosteronism (PA) is the most common form of secondary hypertension. It is caused by overproduction of aldosterone by either a unilateral aldosterone-producing adenoma (APA) or by bilateral adrenal hyperplasia (BAH). Distinction is crucial, because PA is cured by adrenalectomy in APA and is treated by mineralocorticoid receptor antagonists in BAH. The distinction is currently made by adrenal vein sampling (AVS). AVS is a costly, invasive and complex technical procedure with limited availability and is not superior in terms of outcomes to CT scan-based diagnosis. Thus, there is a need for a cheaper, non-invasive and readily available diagnostic tool in PA. We propose a new diagnostic imaging modality employing the positron emission tomography (PET) tracer [^{68}Ga]Ga-PentixaFor. This tracer has high focal uptake in APAs, whereas low uptake was shown in patients with normal adrenals. Thus, [^{68}Ga]Ga-PentixaFor PET/CT is an imaging modality with the potential to improve subtyping of PA. It is readily available, safe and, as an out-patient procedure, much cheaper diagnostic method than AVS.

Methods and analysis We present a two-step randomised controlled trial (RCT) protocol in which we assess the accuracy of [^{68}Ga]Ga-PentixaFor PET/CT in the first step and compare [^{68}Ga]Ga-PentixaFor PET/CT to AVS in the second step. In the first step, the concordance will be determined between [^{68}Ga]Ga-PentixaFor PET/CT and AVS and a concordance probability is calculated with a Bayesian prediction model. In the second step, we will compare [^{68}Ga]Ga-PentixaFor PET/CT and AVS for clinical outcome and intensity of hypertensive drug use defined as daily defined doses in a RCT.

Ethics and dissemination Ethics approval was acquired from the medical ethical committee East-Netherlands (METC Oost-Nederland). Results will be disseminated through peer-reviewed articles.

Trial registration number NL9625.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ We will use a two-step study design that consists of a feasibility study in the first step, followed by a randomised outcome-based diagnostic trial in the second step if the first step yields predefined positive results, which allows modifications to the trial after its initiation without undermining its validity.
⇒ This study responds to the urgent need for an accessible, non-invasive alternative to adrenal vein sampling (AVS) in the diagnostic work-up for patients with primary aldosteronism.
⇒ Even though AVS is considered the gold-standard diagnostic, it may still incorrectly subtype primary aldosteronism, which is a limitation in the first step of this study.

INTRODUCTION

Primary aldosteronism (PA) is the most common form of secondary hypertension, affecting an estimated 2.4% (200 000 patients) of the hypertensive population in the Netherlands, with an annual incidence of around 5.000.[1 2] Although this has previously been a vastly underdiagnosed aetiology of hypertension, the number of recognised patients with PA is increasing due to higher awareness among internists and primary care physicians. PA has a major impact on healthcare due to a considerably higher cardiovascular morbidity, which results in increased risk of strokes, atrial fibrillation and heart failure (2.58, 1.77 and 2.52 times higher, respectively) as compared with essential hypertension.[3 4] PA is caused by overproduction of aldosterone by either a unilateral aldosterone-producing

adenoma (APA) or by BAH.[5] Proper distinction between the two ('subtyping') is crucial, because APA is cured by adrenalectomy and BAH is treated by specific mineralocorticoid receptor antagonists (MRAs).[5] In comparison with lifelong medical treatment, adrenalectomy in patients with APA improves patients' quality of life (QoL), reduces their cardiovascular risk and has substantial costs benefit.[4 6] We aim to subtype all patients with PA more efficiently and reliably for accurate diagnosis and individually optimised therapy.

The distinction between unilateral and bilateral PA is currently made either by selective aldosterone secretion measurement with (invasive) AVS or by adrenal CT scan. CT scanning is readily available and cheap compared with AVS, but AVS has the advantage of obtaining a functional diagnosis in CT-identified nodules and can uncover APAs below the detection-range of a CT scan. Therefore, AVS has emerged as the Endocrine Society guidelines reference standard for PA subtyping.[5 7–9] Nonetheless, AVS is invasive, expensive, requires considerable technical skills and carries a risk of serious complications.[10] AVS is an in-hospital procedure that is available in five centres only in the Netherlands, which in view of the increasing incidence of PA, hinders compliance with the guidelines. Moreover, we have demonstrated in the SPARTACUS trial that it is in fact not superior in terms of outcomes to CT scanning with respect to correct localisation of APAs, the pivotal prerequisite for successful curative operation.[11] Therefore, AVS results in extra healthcare costs that cannot be justified by proportional improvements in outcome and QoL of patients with PA.

Responding to the urgent need for a non-expensive, safe diagnostic for subtyping PA, we propose a diagnostic imaging modality that provides functional information allowing improved diagnostic accuracy as compared with AVS. In recent years, chemokine receptors in human cancers have emerged as particular targeting focus.[12] One of these receptors is C-X-C chemokine receptor type 4 (CXCR4), which binds the CXC chemokine stromal cell-derived factor-1. The positron emission tomography (PET) tracer [^{68}Ga]Ga-PentixaFor PET/CT is a specific ligand for CXCR4 showing promising diagnostic results in several malignancies.[12] Furthermore, the risks associated with the tracer injection are negligible and adverse reactions to [^{68}Ga]Ga-PentixaFor have not been observed.

Two recent case series demonstrate the implementation of [^{68}Ga]Ga-PentixaFor PET/CT in patients with adrenal lesions. Both describe high expression of CXCR4 in aldosterone-producing tissue.[13 14] The most recent case series performed [^{68}Ga]Ga-PentixaFor PET/CT specifically in patients with a suspicion of PA. High focal uptake of [^{68}Ga]Ga-PentixaFor in APAs was found, whereas substantially lower uptake was shown in patients with non-functional adenoma and BAH.[14] The paper also describes a sensitivity, specificity and accuracy of [^{68}Ga]Ga-PentixaFor PET/CT in distinguishing APA by visualisation of 100%, 78.6% and 92.3%, respectively.[14] An interesting question, which is not answered in both papers, is whether [^{68}Ga]Ga-PentixaFor PET/CT can detect the dominant side in patients with bilateral hyperplasia. We can only speculate that this is the case, since the proportion of CYP11B2 positive cells is positively correlated with the proportion of CXCR4 positive cells.[13 14] The SUV_{max} is positively correlated with the proportion of CXCR positive cells.[14] Taken together, we can assume that the dominant side of bilateral hyperplasia has a higher proportion of CYP11B2 positive cells and CXCR4 positive cells compared with the contralateral side, which may result in a higher SUV_{max} seen on the PentixaFor PET/CT. Altogether, [^{68}Ga]Ga-PentixaFor PET/CT is a new functional imaging modality with the clear potential to tackle the formidable challenge of improving subtyping of PA and accurately localising APAs. Furthermore, it is readily available in most centres where PET scanning is available and it seems to be accurate, safe and, as an out-patient procedure, at much lower costs than AVS.

In order to establish [^{68}Ga]Ga-PentixaFor PET/CT as a standard in subtyping PA, two questions need to be addressed. (1) Is [^{68}Ga]Ga-PentixaFor PET/CT accurate in subtyping PA? (2) Does management of patients with PA based on [^{68}Ga]Ga-PentixaFor PET/CT result in comparable or improved clinical outcomes as compared with AVS? We have designed a novel, adaptive two-step study protocol that efficiently addresses these two research questions. In the first step, we will assess the accuracy of [^{68}Ga]Ga-PentixaFor PET/CT compared with AVS. Accuracy is defined as concordance of [^{68}Ga]Ga-PentixaFor PET/CT with AVS in subtyping patients with PA with either unilateral or bilateral adrenal aldosterone-overproduction. If the study reaches a predefined threshold of concordance with sufficient certainty, the second study will follow. In this second step, we will compare [^{68}Ga]Ga-PentixaFor PET/CT and AVS in a diagnostic randomised controlled trial (RCT). The primary outcome is the daily defined doses (DDD) of antihypertensive drugs for blood pressure regulation after 1 year, and secondary outcomes are QoL, biochemical and clinical cure (according to the PASO criteria) and costs.[15]

OBJECTIVES

Primary objectives

- To assess the concordance between [^{68}Ga]Ga-PentixaFor PET/CT and AVS for identification and/or lateralisation of APAs in patients with PA (Step 1)
- To assess the quantity of antihypertensive medication after 1 year of follow-up needed to normalise blood pressure in patients who have been managed for PA according to either [^{68}Ga]Ga-PentixaFor PET/CT or AVS defined as DDD. (Step 2).

Secondary objectives

- To establish definitive quantitative criteria of [^{68}Ga]Ga-PentixaFor uptake in unilateral and bilateral PA (Step 1). In patients who receive unilateral adrenalectomy, we compare [^{68}Ga]Ga-PentixaFor uptake in

PET/CT imaging between immunohistochemically (CYP11B2 staining) diagnosed multinodular hyperplasia and solitary adenomas (Step 1, Step 2).
- Biochemical and clinical outcomes according to PASO criteria.[15]
- Cost analysis of [^{68}Ga]Ga-PentixaFor PET/CT versus AVS management (Step 2). Changes in QoL assessed by a validated disease specific health-related Quality of Life (HRQoL) questionnaire and the Short Form health survey (SF36)[16 17] (Step 2). To perform a safety analysis of [^{68}Ga]Ga-PentixaFor administration on clinical symptoms by adverse events outcomes (Step 1).

METHODS/DESIGN

Study design

We propose a two-step study design that in the first step consists of an adaptive feasibility study to assess the concordance between [^{68}Ga]Ga-PentixaFor PET/CT and AVS, followed by a randomised outcome-based diagnostic trial in the second step, if the first step yields predefined positive results. The novelty of our study is the adaptive two-step design, which allows modifications to the trial after its initiation without undermining its validity and integrity. This makes the study flexible and more sustainable and saves time by combining both steps in one trial.

Participants

In order to be eligible to participate in this study, a subject must meet all of the following criteria:
- Patients with PA, confirmed by an elevated aldosterone/renin ratio (ARR) and an intravenous salt-loading test (according to the Endocrine Society guidelines).
- The patient is ≥18 years of age at the time of consent.
- The patient has provided written informed consent authorisation before participating in the study.

A potential subject that meets any of the following exclusion criteria will be excluded from participation in this study:
- Refusal by the patients to undergo[^{68}Ga]Ga-PentixaFor PET/CT, AVS, CT, or adrenalectomy.
- Suspicion of familial hyperaldosteronism type 1 (FH-1) or type 3 (FH-3).
- Suspicion of adrenocortical carcinoma.
- Severe comorbidity potentially interfering with treatment or HRQoL.
- Requirement of medication interfering with the study protocol.
- Any medical condition present that in the opinion of the investigator will affect patients' clinical status.
- Pregnancy or lactation.
- Estimated glomerular filtration rate <40 mL/min/1.73 m^2.
- Interfering treatment in between [^{68}Ga]Ga-PentixaFor PET/CT and AVS.

Intervention, comparator and outcomes

First step

The diagnosis of unilateral or bilateral disease will be based on AVS. Subsequently, in these patients, a [^{68}Ga]Ga-PentixaFor PET/CT will be performed. Patients will receive a single intravenous injection of 150±50 MBq [^{68}Ga]Ga-PentixaFor. A dynamic PET/CT scan will be acquired for 60 min after injection in the first 10 study participants followed by a static abdominal PET scan 2 hours post injection to determine the optimal time point for imaging. A single static abdominal PET/CT scan will be obtained in all following subjects at the time point considered optimal. The [^{68}Ga]Ga-PentixaFor PET/CT images will be interpreted by a clinician, blinded for the AVS and CT results, with extensive experience in radiolabelled imaging. Lateralisation (based on the SUV) will be compared with lateralization results of AVS. Based on the results of AVS, patients will undergo unilateral adrenalectomy or MRA therapy (standard of care). Six to 12 months after surgery, an ARR will be performed to investigate clinical outcome and biochemical remission.[18]

Second step

In a RCT, patients with PA will undergo either [^{68}Ga]Ga-PentixaFor PET/CT or AVS/CT. Each subject will be randomly assigned to one of the diagnostic methods. Based on [^{68}Ga]Ga-PentixaFor PET/CT or AVS results, patients with a unilateral indication of PA will receive adrenalectomy and patients with a bilateral cause of PA will receive MRAs (figure 1). Both diagnostic methods will be compared by measuring the DDD of antihypertensive medication in both groups after 1 year of follow-up starting at time of diagnosis. Management of antihypertensive medication will be compared between groups in terms of DDD. Secondary outcomes are biochemical cure, QoL and a cost analysis.

Patient and public involvement statement

The Dutch adrenal patient organisation (Bijniervereniging NVACP) endorses this project and have sent their letter of approval. We strive to establish a patient advisory board accompanying the course of the study from a patient perspective. We will actively involve a patient representative in the protocol design of the RCT.

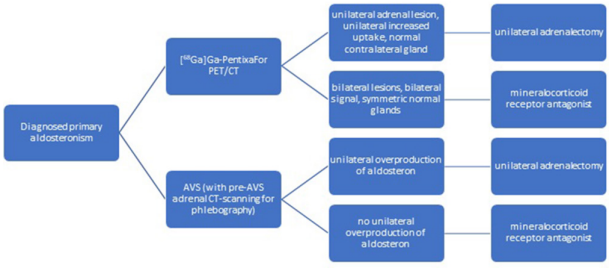

Figure 1 Randomisation and treatment of patients with PA in the second step. AVS, adrenal vein sampling. PA, primary aldosteronism.

Study procedures

Screening visit

The goal of the screening visit is to review inclusion and exclusion criteria, review demographic information and medical history and obtain written informed consent. Site staff will record the subject's medical history in the subject's source records and transcribe the information onto the medical history section of the Case Report File (CRF) in Castor. Written informed consent will be obtained from the patients after adequate explanation of the aims, methods and potential hazards of the study and prior to the initiation of any study-related procedures (see online supplemental data model consent form step 1 and 2). This will be done according to the guidelines provided in the Declaration of Helsinki, ICH E6 Guideline for Good Clinical Practice and requirements of Title 21 CFR 50.20 through 50.27. The subject must be made aware and agree that personal information may be scrutinised during an audit by competent authorities and properly authorised persons. However, personal information will be treated as strictly confidential.

Adrenal vein sampling

Before the AVS procedure, interfering antihypertensive agents will be stopped with an interval of 4–6 weeks for MRAs and potassium sparing diuretics, and 2 weeks for ACE-inhibitors, angiotensin receptor-blockers, diuretics and beta-blockers. In case of uncontrolled hypertension, treatment with calcium-blockers, doxazosin or hydralazine will be allowed during diagnostic workup. We admit patients the day prior to AVS for timely correction of hypokalaemia if present. Hypokalaemia is corrected with oral or intravenous potassium to reach a serum potassium level ≥3.5 mmol/L. AVS will be performed after at least 3 hours of recumbent position. It will be performed under continuous cosyntropin stimulation of 50 µg/hour starting 30 min before the procedure. The adrenal veins will be catheterised by a percutaneous femoral vein approach. Catheter tip position will be confirmed by injection of a small amount of contrast. Blood samples are obtained sequentially by gravity or gentle negative pressure. During the procedure, correct catheter position is verified by cortisol measurements. In case of incorrect catheter position, new samples will be obtained within the same sampling procedure. Formulas and cut-offs for selectivity and lateralization are detailed in figure 2.

CT-scan

We will perform CT-scans with a 64-row multidetector CT-scanner, with reconstruction on 1 mm slices and with the following parameters: 32×0.6 mm detector, 120 kVp, 200–250 mAs (effective), 370 ms rotation time. Reconstructions of 0.75×0.5 mm and 3×3 mm will be performed. CT images are interpreted by a radiologist. In case of an adrenal lesion with an attenuation of less than 10 HU in a lesion smaller than 4 cm on unenhanced images, an adenoma is the most likely diagnosis. For lesions with attenuation of more than 10 HU and larger than 4 cm, we will perform contrast series with 100 mL of intravenous contrast (300 mg/mL) with a flow of 4 mL per second for better characterisation of the lesion. We will use bolus tracking with a 100 HU threshold and a post-threshold delay of 40 s, resulting in a delay of 60 s after injection. CT images will be acquired 60 s and 15 min after contrast infusion. We will use an absolute percentage washout >60% or a relative percentage washout >40% as a cut-off to confirm the diagnosis of an adrenal adenoma.

	Formula	Cut-off
Selectivity index	$\text{Cortisol}_{\text{adrenal vein}} / \text{Cortisol}_{\text{iliac vein}}$	≥ 3·0
Lateralisation index	$\frac{[\text{Aldosterone}_{\text{dominant}}/\text{Cortisol}_{\text{dominant}}]}{[\text{Aldosterone}_{\text{non-dominant}}/\text{Cortisol}_{\text{non-dominant}}]}$	≥ 4·0
Suppression index	$\frac{[\text{Aldosterone}_{\text{non-dominant}}/\text{Cortisol}_{\text{non-dominant}}]}{[\text{Aldosterone}_{\text{iliac vein}}/\text{Cortisol}_{\text{iliac vein}}]}$	≤ 1·0

Figure 2 Formulas and cut-offs for selectivity, lateralisation and suppression index in AVS. AVS, adrenal vein sampling.

[^{68}Ga]Ga-PentixaFor synthesis

Development of [^{68}Ga]Ga-PentixaFor was performed according to the procedures of the Department of Medical Imaging and Nuclear Medicine of the Radboud University Medical Center. The precursor (PentixaFor-acetate) is produced by PentixaPharm GmbH (Berlin, Germany). The final radiopharmaceutical is composed of a 10.5 mL solution of [68Ga]Ga-PentixaFor and PentixaFor acetate in Phosphate-buffered saline (PBS)/0.9% saline with ascorbic acid and up to 5% ethanol. The radiopharmaceutical solution is obtained using an excess of the precursor substance PentixaFor, an automated synthesis device and a commercially available synthesis kit for chelator-conjugated peptides. The radiolabelling steps consisted of (1) 68Ga elution from a 68Ge/68Ga generator and purification/concentration through an ion-exchange cartridge; (2) heating of precursor PentixaFor and 68Ga in HEPES buffer at 100°C for 10 min; (3) and purification by reversed-phase solid phase extraction using ethanol and (4) sterile filtration prior final formulation by dilution with ascorbic acid in 0.9% saline/PBS, in order to draw patient doses of approximately 100–200 MBq and a maximum of 50 µg at the time of administration. The radiochemical purity exceeded the acceptance criteria of ≥95%.

[^{68}Ga]Ga-PentixaFor PET/CT

Patients will receive [^{68}Ga]Ga-PentixaFor in a single intravenous dose. The radiopharmaceutical will be injected through the indwelling intravenous catheter. Following completion of the [^{68}Ga]Ga-PentixaFor injection, a normal saline flush (approximately 10 mL) will ensure that all [^{68}Ga]Ga-PentixaFor remaining in the infusion line is injected. The estimated radioactive dose will be determined by measuring the amount of radioactivity in the syringe preinjection and postinjection, using an appropriately calibrated radioisotope dose calibrator in accordance with the nuclear medicine department's standard operating procedures (SOPs). The injected activity will be recorded in the subject's source records and

transcribed into the CRF. Any administration complication of the drug (eg, overdose, observable extravasation, medication error) will be reported to the monitor within 24 hours of the event and will be recorded in the subject's source records and transcribed onto the CRF. If the PI determines an adverse event occurred after injection or because of extravasation, this will be reported.

A dynamic scan of the abdomen will be obtained, using a Siemens Biograph mCT time-of-flight PET/CT camera for 60 min post injection and a static scan will be taken 2 hours post injection in the first 10 study participants to determine the optimal time for scanning (based on the adrenal to background ratio and the ratio between the adrenals). The dynamic images will be reconstructed using the following scheme: 1×40 s, 10×5 s, 3×10 s, 2×15 s, 5×30 s, 5×120 s, 5×300 s and 2×600 s. All following study participants will undergo an abdominal static PET/CT scan at the time point after injection considered optimal based on the results obtained in the first 10 individuals. Fused PET and low-dose CT images will be obtained to evaluate uptake of [^{68}Ga]Ga-PentixaFor. Typically, scanning is performed in a supine position with arms positioned above the head during the entire scan to avoid possible CT beam hardening artefacts. Total scanning time of the suggested range will take approximately 5 min using one bed position. Attenuation-corrected (AC) and non-AC images of PET data are reconstructed and automatically fused with low-dose CT data for superior visual analysis of the imaged region.

Image analysis

The [^{68}Ga]Ga-PentixaFor PET/CT images will be interpreted by a clinician, blinded for the AVS and CT results, with extensive experience in radionuclide imaging. A positive PET/CT adrenal lesion detection is visually detected and defined as a lesion with high focal PentixaFor uptake. A negative lesion has lower uptake of PentixaFor. Semiquantitative analyses will be performed with PMOD software (Zurich, Germany: PMOD TECHNOLOGIES). We will use a VOI of 10 mm for adrenal lesions. The optimal scan time will be determined with the use of time activity curves which are based on volume of interests of adrenal lesions and liver on dynamic imaging sequences. Cut-off values for lateralisation of semiquantitative analyses, such as liver to lesion ratio, lesion to contralateral ratio and SUV_{max}, will be defined with the construction of receiver operator curves.

Follow-up

After start of treatment (adrenalectomy or mineral receptor antagonist), all patients will be followed by a strict protocol in an outpatient clinic of the participating centres for 1 year: physicians will be instructed by a guide provided by the clinical record form to add other antihypertensive medication according to a treatment algorithm in case blood pressure readings by a semiautomatic device or home blood pressure measurement are over 135/85 mm Hg. Besides these strict treatment goals and the treatment algorithm, we try to limit therapeutic inertia by the treating physicians with regard to blood pressure by frequent auditing and feedback of participating centres.

The QoL questionnaires already administered at baseline will be repeated at the end of follow-up and at fixed moments in between, that is, immediately after completion of the diagnostic trajectory, immediately after completion of the initial clinical management and at 6 months after inclusion in the trial.

One year after adrenalectomy or start of MRA, blood pressure measurements will be recorded as well as the types of antihypertensive drugs and the intensity of hypertensive drugs used in DDD. In patients that underwent adrenalectomy, we will repeat a salt loading test in order to investigate whether PA has been cured biochemically.

Sample size calculation

Step 1

In the first step, analysis is performed with a Bayesian prediction model. This model allows to recalculate the concordance probability after each included patient. We aim for a concordance of more than 50% with the requirement that the lower limit of the 80% CI is more than 50% and the width of the CI is <0.2. This concordance precondition is based on the concordance of AVS and CT-scan in the literature.[10] Remarkably, the concordance of 50% leads to comparable clinical outcomes in these patients. Once we have sufficient evidence for a concordance probability more than 50% with the predefined accuracy rate, inclusion of patients will be stopped and we will proceed to the diagnostic RCT (figure 3).

To determine the maximum number of patients, we assume that the probability of a concordant result between AVS and [^{68}Ga]Ga-PentixaFor PET/CT equals 50% which should be estimated with an accuracy of 20% and a probability of 80%. We need a number of patients so that the 80% credible interval (Bayesian CI P10 to P90) for a probability of concordance has a maximum interval width of 20%. Based on simulations for such a Bayesian analysis, the maximum number of patients required is 41, which is realistic considering 80 patients from Radboudumc and UMCU undergo AVS on a yearly basis. So, if the 50% concordance probability or the concomitant requested accuracy is not reached after 41 patients, the study will not proceed and the RCT is cancelled. Assuming that the probability of concordance of [^{68}Ga]Ga-PentixaFor PET/CT is a uniform distribution between 50% and 100%, we need, in the ideal situation of 100%

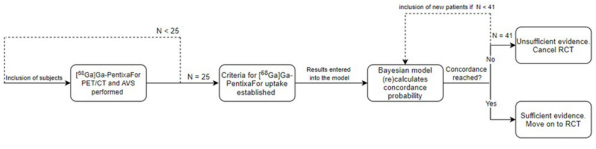

Figure 3 A flowchart diagram which details step 1 and decision making prior to advancing to step 2. AVS, adrenal vein sampling; RCT, randomised controlled trial.

concordance of results, very few patients to find a 80% credible interval that is entirely above 50%. However, we also need a certain number of included patients to establish accurate quantitative criteria of [^{68}Ga]Ga-PentixaFor PET/CT uptake. We chose for a predetermined minimal number of 25 patients, because of the possibility that otherwise the concordance probability will be reached before we can determine accurate quantitative criteria of [^{68}Ga]Ga-PentixaFor PET/CT uptake.

To give an example: if 41 patients are included and the Bayesian analysis results in an 80% credible interval for the concordance probability of (0.42 to 0.65), the RCT will be cancelled. The real probability on concordance might be 0.5 but the requested accuracy is not reached: maximal allowed interval width is 0.2 and here we find 0.23. Other example: we find, after inclusion of patient number 34, an interval of [0.54 to 0.65]. The inclusion stops and the RCT will be started. The requested accuracy for the estimated concordance probability is reached (interval width equals 0.11, where 0.2 is allowed) and we are now for 80% sure that the concordance probability falls between 0.54 and 0.65 (so is larger than 0.5). A last example in which the RCT will be performed: after inclusion of 25 patients, we find the interval [0.51 to 0.62]. Inclusion stops because we are for 80% sure that concordance probability falls between 0.51 and 0.62 and that is sufficiently accurate (interval width equals 0.11, while 0.2 is allowed).

Step 2

In case the first step demonstrates sufficient accuracy, we will continue to the second step, in which we need another sample size calculation for the prospective two-armed RCT. The power calculation for this part of the study is based on the Spartacus data.[11]

The power of this study to detect a difference in the intensity of antihypertensive medication, expressed in DDD, is 84%, based on the following assumptions:

- AVS is the gold standard test.
- APA is diagnosed by AVS in 50% of the cases.[11]
- APA is diagnosed by [^{68}Ga]Ga-PentixaFor PET/CT in 50% of the cases. With AVS as a golden standard, this PET/CT-scan result is incorrect in 33% of the cases (ie, of the 50% APA diagnosis, 33.5% is correct and 16.5% is incorrect). This is based on previous research[19] (figure 4).
- Antihypertensive medication use after 1 year of follow-up, expressed in number of DDD (mean±SD), is 4.85 in patients with BAH and 1.2 in patients that have been operated for APA [4]. We assume that biochemical failure of operation results in the same use of medication as in cases with BAH.
- Mean number of DDD after 1 year follow-up in the [^{68}Ga]Ga-PentixaFor PET/CT group is therefore (33.5×1.2 + 66.5×4.85)/100=3.625 and in the AVS group (50×1.2 + 50×4.85)/100=3.025.
- For the non-inferior trial, the following hypothesis will be used:

H0: [^{68}Ga]Ga-PentixaFor PET/CT is inferior to AVS; [^{68}Ga]Ga-PentixaFor PET/CT results in 1 or more DDD compared with AVS.

H1: [^{68}Ga]Ga-PentixaFor PET/CT is not inferior to AVS; [^{68}Ga]Ga-PentixaFor PET/CT results in less than 1 DDD compared with AVS.

- Calculation of the sample size for a non-inferiority trial and a two-sided significance level of 0.05 to reject H0, indicates a required sample size of 200 patients, with 100 in a group (s=1.0; δ=0.6; α=0.05; β=0.16).
- Taking into account a potential dropout rate of 20%, we aim to include 120 patients in each group.
- The Power calculation will be updated for the results of the first step of the study for a more accurate and current sample size calculation.

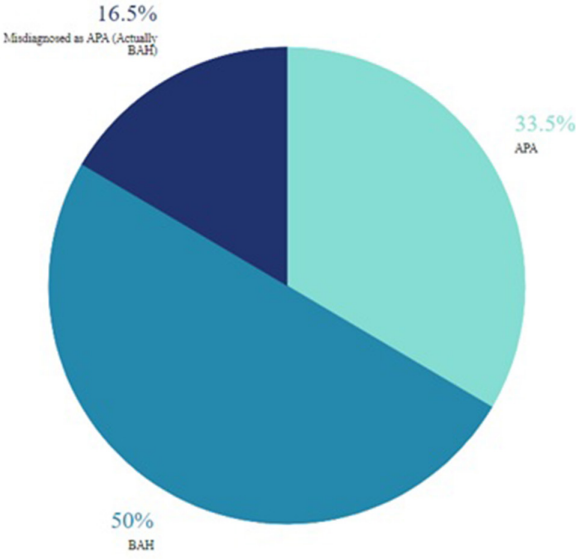

Figure 4 Pie chart of CT managed diagnosis of PA. Assuming 33% of the CT diagnosed APA is incorrect,[19] CT diagnoses APA in 33.5% and BAH in 66.5% of the PA cases. APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia; PA, primary aldosteronism.

ETHICS AND DISSEMINATION

Study approval has been granted by the medical ethical committee East-Netherlands (METC Oost-Nederland) and The Central Committee on Research Involving Human Subjects (CCMO). This two-step clinical study has been registered with the Netherlands Trial register (Trial NL9625). The data will not be used for any purposes other than for the research as described in the protocol. We will publish the final study results in a peer-reviewed journal and present findings at scientific meetings and congresses. The data will be uploaded on an online repository and will be accessible after proper request.

DISCUSSION

This two-step clinical trial is the first trial to assess the accuracy and clinical outcomes of [^{68}Ga]Ga-PentixaFor PET/CT

compared with AVS as a diagnostic modality in PA. This study responds to the urgent need for an accessible, non-invasive alternative to AVS in the diagnostic work-up for patients with primary aldosteronism.

One major strength of this study is the two-step study design. In the first step, we determine the accuracy and quantitative criteria for lateralization of [^{68}Ga]Ga-PentixaFor PET/CT and will use an adaptive Bayesian model to predict outcomes after each inclusion. If the first step reaches the predefined results, we will continue to the second step, a diagnostic RCT. Due to the adaptive two-step design, modifications to the trial are possible after its initiation without undermining its validity and integrity, for example the situation in which the concordance requirements are met in step one and we therefore continue to the second step. This makes the study flexible, saves time and is sustainable, compared with studies in which a fixed number of patients is included.

One limitation of the study is that even though AVS is considered the gold-standard diagnostic, it may still incorrectly subtype PA. When considering the minimal concordance of 50%, one can argue that this concordance limit is low. The SPARTACUS trial demonstrated, even though it had its limitations, that a 50% concordance between CT and AVS had no significant effect on the clinical outcome of patients.[11] We set the predefined 50% concordance for the lower limit of the CI, meaning that we aim for a concordance higher than 50%. Ultimately, the second step of the study will demonstrate what the clinical performance of [^{68}Ga]Ga-PentixaFor PET/CT compared with AVS as a diagnostic modality is.

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