Introducti

‘Autofluorescence’ (AF) was first described in 1838 and represents the emission of light in the near-infrared (NIR) spectrum by a biological substrate, following excitation by light of a certain wavelength [1]. This property has been exploited in several clinical specialties including dermato-

logy, ophthalmology and oncological surgery [2–8]. AF in parathyroid tissue was first demonstrated in vivo in humans in 2011 [9] and has been shown to be distinct from thyroid autofluorescence (around 3 times brighter), typically occurring in the 800–950 nm wavelength (after excitation at around 785 nm). It has therefore been considered as a possible intraoperative adjunct in localisation of the parathyroid glands during neck endocrine surgery [9–11]. This generated considerable interest as the detection of parathyroid tissue intraoperatively is dependent upon visual inspection by the operating surgeon with outcomes being commensurate with experience [12, 13].
Several studies have also sought to use AF with an intravenous fluorophore, such as indocyanine green (ICG), to demonstrate the vascularity of the glands as a surrogate marker of parathyroid function following thyroidectomy, with the hypothesis that its use will prevent post-thyroidectomy hypoparathyroidism [14, 15]. In parathyroidectomy (PTx) for hyperparathyroidism, the challenge is to ensure identification of all parathyroid tissues, pathological and normal, as failure to do so results in persistent hyperparathyroidism. Given that the rate of failure to cure in the first time parathyroid surgery can be as low as 2%, achieving a further reduction would require a significant improvement in either pre- or intraoperative localisation adjuncts. An understanding of the characteristics of AF in physiological and pathological parathyroid tissues is required if it is to be used with benefit. However, although multiple candidates have been proposed, the endogenous fluorophore/s responsible for parathyroid autofluorescence is/are not yet known [1, 9]. The purpose of this study was to explore the potential clinical correlates for parathyroid AF and assess the clinical utility of AF in parathyroid surgery.

**Methods**

The aims of this study were twofold: (1) to assess the reliability of parathyroid AF in surgery for hyperparathyroidism (HPT) and (2) to establish whether a correlation exists between the intensity of AF and any of the following variables—pre-operative serum calcium or parathyroid hormone (PTH), SestaMIBI positivity, gland weight and histological composition of the gland.

Following the approval by the ethical review board, a prospective study was conducted of consecutive patients undergoing parathyroid surgery in a single tertiary referral teaching hospital, performing over 650 endocrine surgical procedures (thyroid, parathyroid and adrenal) per annum. Inclusion criteria were: all patients undergoing parathyroid surgery (bilateral exploration, unilateral exploration and focussed lateral approach) for primary or secondary disease. Primary hyperparathyroidism (pHPT) was diagnosed on the basis of an elevated serum calcium in the presence of an inappropriately unsuppressed PTH or normocalcaemic hyperparathyroidism, a normal calcium and inappropriately high PTH. Urinary calcium/creatinine clearance ratio was calculated from a 24-h urine collection in all patients to exclude benign familial hypocalciuric hypercalcaemia [16]. The indications for intervention in pHPT were clinical symptoms or the loose application of the NIH criteria in asymptomatic disease [17]. Secondary hyperparathyroidism was due to chronic renal failure requiring dialysis in all cases, and the KDIGO guidelines were used to determine the timing of intervention, based on the principle that the severity of disease predicted irreversibility post-transplant [18]. Patients with pHPT underwent dual localisation studies pre-operatively with ultrasound and SestaMIBI. Additional imaging with 4DCT and/or venous sampling/parathyroid angiography was performed in patients with recurrent/persistent pHPT. Those with concordant imaging went on to focussed lateral approach PTx and non-concordant/single modality positive imaging to unilateral exploration with intraoperative PTH estimation (StatIOPTH, Future Diagnostics) guided by the Miami criteria [19]. Unlocalised primary disease and renal HPT were managed with bilateral exploration. There were no exclusion criteria.

The AF device used was the ‘Fluobeam® 800’ (Fluoptics, Grenoble, France), because it is licensed for use in parathyroid surgery, does not require the administration of fluorescent contrast, which carries a risk (albeit low) of contrast allergy/anaphylaxis which was not felt to be justified in a research setting and requires no contact between the camera and tissues (as per the device used by the Mahadevan-Janssen group [9, 20]). The system, as shown in Fig. 1, was used following operative exposure of the parathyroid glands (as the brightness of AF was known to be affected by tissue covering parathyroid glands).

The primary outcomes were: (1) percentage of parathyroid glands showing an AF signal. Secondary outcomes were correlation between the intensity of parathyroid AF and (1) pre-operative serum calcium, (2) pre-operative serum PTH, (3) SestaMIBI positivity and for glands which were excised (4) gland weight and (5) histological composition of the gland. Histology was taken as...
the gold standard for identification of excised glands, whereas surgeon identification was chosen as the gold standard for parathyroids left in situ. Surgeon identification was used to avoid biopsy, which would not otherwise have been performed as part of standard surgical management, to obviate any risk to the patient solely from the study. Surgeon identification was performed by two surgeons, one of whom was a consultant surgeon (FP/NT) and one a senior trainee. The AF equipment used did not permit immediate in vivo quantification and so the intensity of parathyroid AF was graded as low, medium or high by the same two surgeons. This AF ranking was judged in relation to adjacent thyroid tissue with ‘low’ parathyroid AF being regarded as a little brighter than thyroid tissue, ‘medium’—much brighter and ‘high’—very much brighter. Each surgeon reported their assessment to an observer (medical student) who recorded this. A flowchart of the study protocol is shown in Fig. 2.

Standard demographic information, pre- and post-operative biochemistry, results of imaging studies and histopathology including gland weight were collected. Histopathology was considered to be that of the predominant cell type, i.e. oxyphil, chief or clear, as percentage composition was not routinely reported. Statistics were calculated using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Reliability of parathyroid AF was calculated as a proportion of glands positively identified by the surgeon if not excised or histopathology if excised. The Wilcoxon signed-rank test was used to compare the continuous variables of serum calcium, PTH and weight against the ranked intensity of AF and McNemar’s test to compare categorical variables of predominant composition of the excised glands against the intensity of AF.

Results

Over 8 months from February to October 2017, 96 patients underwent PTx and were included in the study. Median age was 59 years (range 24–85), almost three-quarters were female (72F:24M), 87 had pHPT and 9 secondary HPT due to renal failure. Eleven patients had undergone previous surgery for pHPT, and 1, previous surgery for secondary HPT (in other institutions). As shown in Table 1, in the patients with pHPT, median pre-operative serum-corrected calcium was 2.79 mmol/l (range 2.44–3.54, upper limit of normal = 2.6 mmol/l) and PTH 16.7 pmol/l (range 4.5–139, upper limit of normal = 6.4 pmol/l) using Abbott Architect and IMMULITE 2000, Siemens, Llanberis, UK). Sixty-five per cent had positive SestaMIBI scans. In the renal HPT group, median pre-operative serum-corrected calcium was 2.41 mmol/l (2.02–2.56), and PTH was 241 pmol/l (78–571).

As shown in Fig. 3, there were 49 bilateral explorations, 41 unilateral and 6 focussed lateral, such that assuming the presence of 4 glands in each patient, a total of 284 glands should have been visualised at surgery. One hundred and twelve were identified and left in situ having been judged to be non-pathological and 3 glands could not be identified. A total of 172 parathyroid glands were deemed to be abnormal and excised (138 in pHPT and 34 in renal HPT). All of these were histologically confirmed to be parathyroids. Median weight of the excised parathyroids in pHPT was 0.53 g (0.05–45 g), and on histological examination, 7 were hypercellular or large normal parathyroids and 130 abnormal, with 83% predominantly chief cell in composition. In renal HPT, all excised glands were abnormal histologically, with 88% showing chief cell predominance and 12% oxyphil.

In total, 257 parathyroids emitted AF (90.5%) and 27 (9.5%) did not, including the 3 missing glands, which could not be identified using AF. A total of 173 glands (60.9%) emitted a high AF signal, 61 (21.5%) medium and 23 (8.1%) low. There was a statistically significant difference in AF between the primary and secondary glands ($p < 0.05$), but no significant difference between histologically normal and abnormal glands. A statistically significant negative correlation was found between serum calcium and intensity of AF ($p < 0.01$) and between serum PTH and AF in renal HPT only ($p = 0.02$). No significant correlation was found between SestaMIBI positivity ($p = 0.32$), gland weight ($p = 0.24$) or gland composition. In secondary HPT glands, there was a correlation between oxyphil predominance and AF ($p = 0.002$).
Discussion

The presence of AF in just over 90% of glands in this study is equivalent to that shown by Ladurner et al. [11] in their study of 41 parathyroids sought during thyroidectomy, in which 37 showed AF (sensitivity of 90%). However, it is lower than the 97% in 264 glands from an unselected group, in which 100% of the parathyroids in patients undergoing thyroidectomy or PTx for pHPT and 54% in secondary HPT due to renal failure demonstrated AF [21]. This suggests that the intensity of AF may be higher in normal parathyroid tissue. Variables in this study which showed statistically significant negative correlation with AF, i.e., serum calcium and secondary versus primary HPT, may be surrogated for the degree of abnormality of a parathyroid gland. This correlation between calcium and AF and the poor AF of renal-induced HPT has previously been reported by the only other study to examine the relationship between AF and patient/gland variables [21]. Serum PTH would also seem to reflect the degree of HPT and therefore abnormality of a gland; however, correlation with AF did not reach statistical significance except in the

Table 1  Correlations between degree of AF and patient/gland variables

| Variable                  | Primary HPT     | Tertiary HPT    | Correlation?                  |
|---------------------------|-----------------|-----------------|------------------------------|
| Serum corr calcium (median mmol/l) | 2.79 (2.44–3.54) | 2.41 (2.02–2.56) | Inverse correlation p < 0.01 |
| Serum PTH (median pmol/l) | 16.7            | 241 (78–571)    | Inverse correlation renal glands only p = 0.02 |
| Gland weight (median g)   | 0.53            | 0.82 (0.11–4.4) | NS p = 0.24                 |
| Predominant cell type     | 83% chief cell predominant | 88% predominantly chief and 12% oxyphil | NS EXCEPT in renal glands oxyphil—positive correlation p = 0.002 |
| SestaMIBI                 | 65% positive    | Not done        | NS p = 0.32                 |

Continuous numerical variables (serum calcium, PTH, gland weight) were assessed with Wilcoxon’s signed-rank and categorical data (predominant cell type on histopathology and SestaMIBI positivity) with McNemar’s test

Fig. 3 Results, showing total number of glands imaged, disease process and degree of AF present. In total, 9.5% (n = 27) showed no AF signal and 61% (n = 173) a high signal
renal group. This was a small (9 patients, 34 glands) sub-
group but may reflect the same phenomenon that more
abnormal glands demonstrate less AF. However, set against
that is the fact that gland weight did not correlate inversely
with AF.

The absence of a correlation between positivity of
SestaMIBI imaging and AF was unexpected: mitochondrial
contents (NADPH) have been proposed as a fluorophore
and so the authors had anticipated that brighter AF would
correlate with oxyphil predominance (due to their densely
packed mitochondria) and therefore SestaMIBI positivity.
This relationship has not, to our knowledge, previously
been tested. Similarly, a prediction that gland composition
would correlate with AF was not manifested except in the
renal HPT patients in whom oxyphil cells correlated with
more intense AF. The lack of correlation with either Sesta-
MIBI or gland composition overall may reflect which
underlying fluorophore causes the AF, i.e. it may be a
biological compound which is present in both chief and
oxyphil cells, or in another cell type altogether. The
extracellular calcium-sensing receptor has also been sug-
gested as a possible fluorophore: it is present in highest
concentrations in the chief cells and known to be down-
regulated in hyperparathyroidism [22], consistent with the
results of other studies comparing fluorescence intensity in
the parathyroids of patients with hyperparathyroidism as
compared to the non-pathological glands encountered at
thyroidectomy [20] and consistent with the lack of corre-
lation with SestaMIBI in this study. The identification of
the responsible fluorophore is not absolutely essential in
using an AF device clinically. However, further work on
the fluorophore/s and their clinical relevance may improve
its performance and help to select appropriate cases for its
use.

This study used a pragmatic approach to test the use of
AF in a ‘real-life’ situation for which the Fluobeam® and
other devices are designed and marketed. Although not
formally assessed, the Fluobeam® was found to be simple
and extremely quick to use, adding only a few minutes and
the inconvenience of turning the lights down, to the oper-
ation. It also has the benefit of not requiring contact with
tissues or changing the operative workflow, which may be
inconvenient, time-consuming and carries a small risk of
infection/tissue trauma. Care must be taken during imaging
to maintain the optimum distance (20 cm) between device
and tissues so as not to affect the AF. This distance is
affected by the depth of the incision and angle at which the
device is held and requires the operating surgeon to
manoeuvre the device while watching the AF image
onscreen until the best image is obtained [23]. With suit-
able positioning of the device, imaging was possible even
in focused lateral surgery.

The use of surgeon identification of parathyroids as the
gold standard in non-excised glands, may be subjective;
however, this methodology replicates that in other studies
[10, 11] and avoids possible harm to the patient from
unnecessary biopsy of non-pathological parathyroids. The
surgeon raters in this study were experienced consultants
and senior trainees in a high-volume centre performing
over 300 parathyroidectomies per annum. In the study, all
parathyroids resected were confirmed as such on
histopathology, with a cure rate of 100%, and in this
sample of 284 putative glands, only 3 could not be found.
In fact, AF was not able to detect those 3 which could not
be located by a surgeon. On this basis, it would seem
reasonable to assume that these surgeons were a reliable
standard against which to judge the clinical utility of AF.
Surgeon ranking of AF intensity was used as the Flu-
obeam®, like other commercially available devices
[14, 15], and does not offer in vivo quantification. This may
result in subjectivity; however, the intention of the study
was to test the device performance in the setting for which
it is designed, so any subjectivity in the study is a true
reflection of its use in clinical practice. Ex vivo approaches
have been described to quantify AF [23], but these require
post hoc analysis, preventing their output from being uti-
ised to assist with decision-making in surgery.

In this study, there were three ‘missing’ glands which
could not be identified by the operating surgeon and were
not identifiable using NIR. The use of NIR to assist with
finding such glands is a very attractive target for future
work. However, it is currently limited by the ability of
current NIR devices to image structures hidden beneath the
surface of a tissue. Improvement in the penetration of NIR
imaging, even by a few millimetres, could have a signifi-
cant impact on its clinical efficacy. Other areas which
could be addressed include further assessment of the rela-
tionship between AF and patient/parathyroid tissue vari-
able on larger numbers of normal and abnormal
parathyroid glands to establish whether the intensity of AF
is higher in more normal glands. The clinical utility of this
would be to assist with surgical treatment of mild hyper-
parathyroidism secondary to multi-gland hyperplasia, this
often being the more subtle disease and more difficult to
localise intraoperatively.

In conclusion, based on the results of this study that
nearly 10% of glands fail to emit an AF signal sufficient for
the surgeon to recognise it and the failure to detect 3
missing glands; it is unlikely that AF will have any
meaningful impact on cure rates in HPT. Its routine use in
clinical practice for HPT therefore cannot be justified.
However, further improvements in the technology, such as
depth of imaging, and confirmation of the relationship
between AF intensity and normal parathyroid tissue, may

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in future allow NIR imaging to be of assistance in select cases.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Informed consent Informed consent was gained from all individual participants in the study.

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