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Clinical, Immunological and Radiological Features of Endocrine Immune Related Adverse Events

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Abstract

Immune checkpoint blockade is a cancer treatment aimed at restoring and enhancing the ability of the immune system to combat a tumour. A recognised side effect is “collateral” immune damage to healthy tissue, or immune related adverse events (irAEs). Immune toxicity to endocrine glands can be rapid and irreversible and may result in the need for lifelong hormone replacement. A major challenge is identifying which patients will develop endocrine irAEs when treated with checkpoint inhibitors. The role of predictive biomarkers such as HLA type or autoantibodies has not been prospectively evaluated. The possibility of detecting pre-clinical endocrine dysfunction using MRI and PET imaging is described in small case series only. This project aims to 1. Define the clinical and immunological features of checkpoint inhibitor related diabetes, hypophysitis and thyroiditis in contrast to spontaneously occuring endocrine autoimminuty and 2. Explore ways to predict and detect endocrine toxicity early with biomarkers and imaging.

First, I define the phenotype and immune mechanisms underlying checkpoint inhibitor related autoimmune diabetes. It was then relevant to discuss atypical or alternate phenotypes of diabetes and pancreatitis which have emerged over the past 2 years. This chapter concludes with a discussion of potential treatments aiming to reverse islet cell destruction, with a letter to the editor published in response to a case report.

The next focus is the diagnostic evaluation of checkpoint inhibitor related hypophysitis. After hypothesising that the true incidence may be underappreciated, this chapter reviews the clinical, biochemical and radiological features in a cohort of patients monitored closely for this irAE.

The third component of the thesis reviews the incidence and natural history of checkpoint inhibitor related thyroiditis. Defining the natural history provided important information guiding management of the hyperthyroid and hypothyroid phases respectively. This chapter includes a diagnostic accuracy study evaluating the role of FDG-PET/CT as a novel tool in the diagnosis of this irAE.

In defining the natural history and diagnostic features of these three endocrine immune

related adverse events, important recommendations about biochemical screening and the complementary role of routine cancer immaging are made. Importantly, treatment considerations relevant to oncologists and endocrinologists alike are outlined.

Declarations

This is to certify that

(i) The thesis comprises only of original work except where indicated in the preface,

(ii) Due acknowledgement has been made in the text to all other material used,

(iii) No third-party copyright material has been included in the thesis

(iv) The thesis is fewer than 50,000 words in length, exclusive of tables, maps, bibliographies and appendices

Name: Anna Galligan Signature: 

Date: August 2020

Preface

The work presented in this thesis was carried out under the supervision of my primary supervisors, Dr Balasubramanian Krishna Murthy and Professor Thomas Kay at the St Vincent's Institute of Medical Research.

The patients studied in this thesis were under the primary care of the Melanoma Unit at the Peter MacCallum Cancer Centre. The ethics application was submitted by the oncology team under the overarching supervision of Dr Shahneen Sandhu (Head, Skin and Melanoma Services), who acted as a co-supervisor for each manuscript presented.

The structure of this thesis is as follows:

1) Chapter 1, The literature review
2) Chapters 2, 3 and 4 dedicated to diabetes, hypophysitis and thyroiditis respectively
    - Each chapter contains either a publication or a manuscript prepared for submission and peer review
    - According to University of Melbourne guidelines, publications are included in word format, with tables and figures included in their logical position within the text
    - For the two published manuscripts only, references are presented at the end of each manuscript
    - Unpublished data are presented in separate paragraphs within the relevant chapter
    - A short summary of work published in manuscripts where I was not the primary author are included with appropriate reference to the original publication (Appendices 2 and 3).
3) Chapter 5, Conclusions and future directions

4) References
5) Appendices

I was responsible for the following elements of this research:

1) Development of all project conception and methods;
2) Design and production of the REDCap data collection tool utilised for each component of this study;
3) Acquisition of the pharmacy extract and preparation for upload into the data collection platform
4) The majority of the retrospective collection and interpretation of clinical and biochemical data;
5) Analysis of data for each study;
6) Preparation of all manuscripts

The following people have assisted in my research:

1) Dr Amir Iravani, Nuclear Medicine Physician, Peter MacCallum Cancer Centre, who provided considered advice regarding the design of the hypophysitis and thyroiditis studies and performed the retrospective review of endocrine gland appearances on all available FDG-PET/CT scans for a large cohort of melanoma patients.
2) Dr Rodney J Hicks, Director: Molecular Imaging; Head: Molecular imaging and targeted therapeutic laboratory, who acted as the second observer in the interobserver agreement study for the role of FDG-PET/CT in hypophysitis and thyroiditis and provided critical review of these two manuscripts.
3) Dr Arian Lasocki, Radiologist, Peter MacCallum Cancer Centre who reviewed all available CT and MRI imaging for the hypophysitis manuscript, contributed to many

study related discussions and who was the primary author of the pictorial essay summarised in Chapter 3 (Appendix 3).

4) Dr Cherie Chiang, who provided a pathology extract for cortisol and thyroid function tests and referred patients of interest for inclusion in the case series (Publication 1 and Appendix 2.)
5) Dr Roslyn Wallace, Medical Oncology Fellow, Peter MacCallum Cancer Centre, for her assistance in the development of the oncologic data collection tools, particularly tumour, prior treatment and response information and for entry of these variables into the REDCap database.
6) Ms Samantha Ann Yoke Yee Yip, MDRP Student, Melbourne University, who was responsible for acquisition of internal and external thyroid function test results required to calculate time to thyroiditis and duration of the thyrotoxic phase.
7) The co-authors for each paper, who unless otherwise mentioned above have contributed to the revisions of each manuscript in preparation for journal submission.
8) Mrs Katerina Kiburg, Department of Endocrinology and Diabetes, St Vincent's Hospital Melbourne, who provided statistical support for the hypophysitis and thyroiditis manuscripts.

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1. Australian Government Research Training Program Scholarship for subsidising this higher degree
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I would like to thank Associate Professor Shahneen Sandhu, who acted as a co-supervisor on each manuscript and provided the valuable oncology perspective and critical review of each paper. I acknowledge and thank all co-authors, particularly Dr Amir Iravani and Dr Arian Lasocki for their dedication to the imaging components of the study. The endocrinology group, Dr Nirupa Sachithanandan, Dr Cherie Chiang, Professor Peter Colman, Associate Professor John Wentworth and Dr Simon Forehan were a constant sounding board for ideas and discussions.

I am very grateful to my funding sources including the Australian Government Research Training Program Scholarship and St Vincent's Hospital Research Endowment Fund.

It is important to acknowledge the patients involved in this study, without whom there would be no research.

Finally, I would like to thank my family, Sue, Luke, John, Harry, Heather and Dmitry, my most valued advisers.

Chapter 1. The Literature Review

1.1 Cancer immunotherapy

The idea that the immune system may play a role in controlling malignant tumours has been a cornerstone of translational research in cancer therapeutics. Although major leaps in basic science have occurred in the last decade, the beginnings of immunotherapy are much older. Anecdotal reports of spontaneous tumour regression after bacterial infection began to emerge in western medical literature in the early 19th century and prompted trials of injected streptococcus for the treatment of osteosarcoma. Then followed novel advances such as the discovery of interferon (IFN), interleukin-2 (IL-2), cancer vaccines, adoptive cell transfer and targeted immunotherapeutics [1, 2]. These advances along with the link between chronic immunosuppression and the development of new skin cancers and other neoplasms have highlighted the role of the immune system in tumour control and tumour escape.

Immune regulation

Regulatory mechanisms exist to maintain immune responses within an appropriate range to avoid autoimmunity. Central tolerance to self-autoantigens is maintained first by the deletion of autoreactive T and B cells from the immune repertoire during lymphocyte development. Peripheral tolerance provides an additional layer of protection against autoimmunity, mediated through a variety of checks and balances including a large repertoire of co-stimulatory and co-inhibitory receptors. Inhibitory receptors or “checkpoints” play an important role in tuning and activating signals to ensure self-tolerance and resolution of the immune response[3].

Immune checkpoints come into play during both initiation and maintenance of T cell activation. The naïve T cell requires a two-step mechanism before undergoing activation and

expansion. Antigen in the form of short peptides is presented by an antigen-presenting cell via the major histocompatibility complex (MCH, referred to as Human Leukocyte Antigen (HLA) in humans) class II molecule for $CD4^+$ T cells and MHC class I for $CD8^+$ T cells, which binds to the T-cell receptor (TCR). A second signal, co-stimulation, classically involves the interaction between the B7 ligands (CD80 and CD86) on antigen presenting cells with CD28 on T cells and is critical for T-cell activation. Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is an inhibitory receptor on the T cell surface which competes with CD28 to bind with B7 ligands with higher affinity, inhibiting naïve T-cell activation. Further downstream, chronically activated T cells can be restrained by the interaction between the inhibitory receptor Programmed cell death protein 1 (PD-1) on the T cell surface with its ligands (PD-L1 and PD-L2) on epithelial and myeloid cells. This interaction provides additional protection against autoimmunity but can be hijacked to allow persistence of chronic viral infections and tumours[4].

Dysfunctional or "Exhausted" T cells

Chronic functional impairment of CD8+ T cells was described in two landmark papers investigating the role of reduced effector function in chronic viral states[5, 6]. In 1998, Zajak and colleagues found that, in mice chronically infected with lymphocytic choriomeningitis virus (LCMV), virus-specific CD8+ T cells expressing activation and memory markers persist indefinitely but are unable to mount sufficient effector responses to clear the infection. The duration of chronic antigen persistence was directly proportional to T cell dysfunction. The finding that chronically activated T cells could continue to exist in a dysfunctional state at high frequencies in these mice was later replicated in human infections with chronic human immunodeficiency virus (HIV) and viral hepatitis, and raised the question of whether strategies to restore effector function may be a successful antiviral treatment[6, 7]. 8 years

later, Barber and colleagues expanded on this same model of LCMV infected mice, tracking CD8+ T cell responses after introduction of viral strains which induced acute and chronic infections respectively. Highly efficient memory T cells proliferated in the mice that cleared the acute infection and developed persistent immunity. In mice with chronic LCMV, the initially activated virus-specific T cells significantly upregulated PD-1 which was associated with impaired cytokine production and proliferative capacity. PD-1 ligand (PD-L1) was also upregulated on chronically infected cells in the mice. The authors then demonstrated that injection of monoclonal antibodies against PD-L1 and PD-1 lead to significantly increased effector T cell functions with increased ability to produce interferon gamma (IFN-y) and tumour necrosis factor alpha (TNF-a) and led to viral clearance[5]. Although these two studies were pre-clinical, they set the scene for translational research into immunotherapeutic targeting of checkpoint receptors.

Like viruses, malignant tumours co-opt immune tolerance mechanisms to evade immune detection and destruction. In the setting of chronic tumour antigen, activated T cells upregulate inhibitory receptors and become dysfunctional or "exhausted." Around the same time that the role of PD-1 was first studied in chronic viral infection, a series of publications emerged describing initiation of an immune response to established tumours after injection with antibodies blocking the CTLA-4 receptor and later, by PD-1 blockade[8]. Cancer immunotherapy with immune checkpoint inhibition (ICI) is based on the premise that an enhanced immune system can recognise and eradicate cancers and the concept of checkpoint blockade comes from the idea that like in LCMV mice, T-cell exhaustion is a reversible or "drug-able" phenomenon in tumours which have escaped the immune response.

Monoclonal antibodies against inhibitory receptors

Monoclonal antibodies against the inhibitory receptors CTLA-4 and PD-1 can restore or re-invigorate exhausted T cells, releasing the breaks on the immune system to combat the tumour. Ipilimumab, the first antibody against CTLA-4 was approved by the US Foods and Drug Administration (FDA) in 2011 and at the time was the first drug to ever demonstrate a survival benefit in advanced melanoma. Soon after, antibodies against PD-1 (pembrolizumab and nivolumab) and PD-L1 (atezolizumab, durvalumab and avelumab) followed in phase III trials[8]. Checkpoint inhibitors have since revolutionised cancer treatment, demonstrating unprecedented survival benefit in melanoma and other malignancies such as non-small cell lung cancer, head and neck squamous cell carcinoma, renal cell carcinoma and triple negative breast cancer[2, 9].

Immune toxicity relating to checkpoint inhibition

While in tumour immunology co-inhibitory receptors are considered detrimental, they are important to tune and shape activating signals to ensure self-tolerance and resolution of the immune response. T-cell exhaustion may be an important way of disarming self-reactive effector memory T cells. By transcriptomic profiling of T cells from patients with autoimmunity, it has been found that T-cell exhaustion signatures are associated with protection from autoimmunity[10]. A disruption in the functioning of inhibitory receptors with checkpoint blockade can result in an unchecked immune response and collateral damage to healthy tissue, i.e. immune related adverse events (irAEs). The remainder of this literature review relates to immune related adverse events, with a focus on endocrine toxicity.

1.2 Immune related adverse events: mechanisms, outcomes and treatment

Tumour response outcomes after immune checkpoint inhibition (ICI) are offset by irAEs, which can occur in almost every organ system. Common Terminology Criteria for Adverse

Events (CTCAE) defined by the United States Department of Health and Human Services National Cancer Institute, provides a standardised grading system for irAEs utilised world-wide to assess severity and guide treatment choices. Scoring ranges from Grade 1 (asymptomatic or mild symptoms only) to Grade 5 (death related to irAE). Grades 3 and 4 are considered severe and life threatening respectively, and may necessitate temporary or permanent treatment cessation of ICI.[11]

A differential adverse event profile between types of ICI

The frequency and phenotype of irAEs differ between blockade of CTLA-4 and PD-1 and are most pronounced with combination immune checkpoint inhibition (cICI). In the landmark Checkmate 067 study, irAEs of any severity occurred in 86% of patients treated with ipilimumab (anit-CTLA-4), 86% of patients treated with nivolumab (anti-PD-1) and 96% in patients treated with combination ipilimumab and nivolumab (cICI). Severe Grade III and IV adverse events occurred in 28%, 21% and 59% respectively[9]. While both CTLA-4 and PD-1 inhibition can induce multi-organ toxicity, certain types of irAEs such as hypophysitis and colitis are more common with CTLA-4 inhibition while type 1 diabetes, thyroiditis and pneumonitis occur more commonly with PD-1 inhibition[4]. The mechanisms behind the differential tumour response and adverse event profile between checkpoint inhibitors are not fully understood.

Both CTLA-4 and PD-1 inhibition can lead to activation and proliferation of T cells, increased antibody production and cross reactivity of a primed immune response to antigens shared by tumour and healthy tissue[4]. CTLA4 primarily functions to regulate T-cell activity at sites of T-cell priming (e.g., secondary lymphoid organs) while PD-1 is expressed upon activation of T cells and PD-L1 is widely expressed in nonlymphoid tissues. The PD-1/PD-

L1 regulatory system is induced by immune signals to attenuate local T-cell responses and thus acts primarily to dampen T-cell activation in the periphery to minimise tissue damage. Upon engagement with PD-L1, PD-1 transmits a negative costimulatory signal through the tyrosine phosphatase to attenuate TCR and CD28 signalling and thus T-cell activation[3].

The differential functions of CTLA-4 and PD-1 receptors can be further characterised by models of spontaneously occurring immunodeficiency and in knock-out mice. Patients with CTLA-4 deficiency experience wide-spread systemic immune cell infiltration and severe multi-organ autoimmunity. CTLA-4 knockout mice develop high titres of autoantibodies and die of lymphoproliferative disorder within 3-4 weeks[12]. After pharmacological CTLA-4 blockade, IrAEs can be systemic and severe. CTLA-4 inhibition appears to have a dose-dependent effect on immune toxicity, with a higher incidence of severe irAEs in patients receiving 10mg/kg compared to 3mg/kg (37 versus 18% in phase III studies)[13]. Mice deficient in PD-1/PDL-1 develop more specific autoimmune syndromes much later in life[14]. After pharmacological blockade of this pathway, irAEs are still common but are generally less severe[9].

The role of regulatory T cells (Tregs)

Tregs are an important subset of T cells which have a predominantly immunosuppressive effect in maintaining self-tolerance. CTLA-4 is constitutively highly expressed on Tregs as is PD-1 to a lesser extent, however their role in Treg function is not well understood. Preclinical studies have demonstrated that germline absence of CTLA-4 in Tregs leads to aberrant T-cell activation and lethal autoimmunity in infant mice[15]. However, CTLA-4 deleted Tregs in adult mice surprisingly maintained a heightened immunosuppressive function with associated resistance to autoimmunity[16]. The CTLA4 inhibitors ipilimumab

and tremelimumab bind to CTLA-4 on Tregs but do not deplete Tregs in the tumour [17]. Modifying the monoclonal antibody to promote depletion of Tregs in addition to attenuating signals via CD28 may improve clinical efficacy with respect to tumour response and increase autoimmune side effects or may deplete the effector T cells.

Shared epitope between tumour and healthy tissue

Another important mechanism behind irAEs is shared antigen reactivity between tumour and healthy tissue. Rapisuwon and colleagues elegantly described this phenomenon in 2019 in their report of a patient with metastatic uveal melanoma treated with cICI. After presenting with extensive metastatic disease nine months after resection of the primary tumour, the patient was treated with two cycles of ipilimumab and nivolumab before developing severe irAEs including vitiligo, retinitis, ocular autoimmune syndrome and duodenitis. Staging imaging three months after starting treatment demonstrated a near complete tumour response in liver, bone and pulmonary metastases. The investigators performed molecular and immunologic analysis on the primary tumour and the liver metastasis and found expansion of identical $CD8^+$ tumour infiltrating lymphocytes in both tissues. Furthermore, the same T cell clone was found in peripheral blood mononuclear cells and in biopsy tissue from the duodenum after the diagnosis of enteritis. The authors conclude that an exceptional T cell response occurred against an antigen common to the uveal melanoma, the healthy ocular tissue, the skin and the duodenum causing both early tumour response and systemic irAE[18]. Robust clonal expansion of shared T cell receptors has also been described in ICI related myocarditis. Immunohistopathological profiling was performed at autopsy on two patients who developed fatal myocarditis after combination ipilumumab and nivolumab. High frequencies of shared clones in the tumour, cardiac and skeletal tissue were found. When comparing tumour specimens from before and after treatment, a near 4-fold expansion in

these T cell infiltrates was observed indicating a marked response[19]. Such mechanistic studies are limited by small numbers in individual reports, however they support the hypothesis that, in the presence of a significant anti-tumour response, a shared epitope between tumour and certain healthy tissues may exacerbate some irAEs.

IrAEs and tumour response

Following from these observations, interest in whether immune toxicity may serve as a biomarker for tumour response has prompted several studies looking for a correlation between them. New onset vitiligo began to emerge in patients with regression of melanoma treated with prior forms of immunotherapy before the advent of ICI. As immunologically opposite entities, regression of melanoma and vitiligo both represent immune-mediated destruction of cells that express melanin. In 2015, a systematic review of a combination of immunotherapeutics including immune stimulation, cancer vaccine and adoptive transfer in 5737 patients found that interval development of vitiligo on treatment was associated with improved overall survival (hazard ratio [HR] 0.25; 95% confidence interval [CI], 0.10-0.61; $p<0.003$)[20]. A difficulty in studies investigating the relationship between ICI and vitiligo are treatment factors including prior exposure to other immunotherapeutics and variable dosing schedules. Nakamura and colleagues performed a multicentre retrospective study of melanoma patients receiving single agent, fixed dose PD-1 inhibition and found that vitiligo was associated with an improved progression free survival (HR 0.24, 95% CI, 0.11-0.55 $p = 0.005$) and overall survival (HR 0.16; 95% CI, 0.03-0.79; $p=0.04$). This study had several limitations. Although the authors reviewed all patients treated at two major centres over a three-year period, only 35 patients were included in the analyses. Vitiligo occurred at a mean time of 5.2 months from treatment commencement, meaning it could not be considered a clinically useful early marker of response. In theory, patients who did not survive past 5

months may not have had time to develop vitiligo. To address potential guarantee-time bias, the authors performed a 20-week landmark analysis, in which the correlation between vitiligo and response rate was no longer statistically significant[21].

In two local studies here in Victoria, rheumatological irAEs in melanoma patients were associated with a higher response rate to PD-1 inhibition, even when the irAE required treatment with prolonged immunosuppression (usually in the form of glucocorticoids, and in some, other disease modifying agents)[22, 23]. The major limitation of the first study was that it was a retrospective case series and the total number of patients with melanoma was not available. The second study was a larger study of 244 patients treated with a PD-1 inhibitor in one major cancer centre in a 6-month period, and even in this sort time frame a strong correlation was established. The association between rheumatic irAEs and tumour response has not yet been replicated in large randomised controlled trials (RCTs).

Endocrine toxicity did not occur in large enough numbers to be associated with tumour response in most phase III studies, however one retrospective analysis of 91 patients treated with PD-L1 inhibition found an adjusted mortality HR of 0.49 (CI 0.25-0.99) and a significantly lower proportion of patients who died in those with thyroiditis[24].

The largest study to date investigating this relationship comes from a secondary analysis of the double-blind RCT EORTC 1325/KEYNOTE-054 clinical trial comparing pembrolizumab therapy and placebo in 1019 melanoma patients. The authors described that the composite of all documented irAEs was associated with a longer progression free survival in the pembrolizumab group. This relationship appears to have been driven by endocrine irAEs, while other individual organ irAEs including vitiligo were not associated with improved

response[25]. Definitive prospective trials with a pre-specified intent to analyse the relationship between irAEs and tumour response are required to corroborate the findings in this group of smaller studies.

Risk factors and biomarkers for immune toxicity

While treatment factors including the type and dose of ICI lead to different side-effects, there are important patient factors also at play [13]. People with a history of autoimmune disease were excluded from the early clinical trials but are not barred from receiving ICI in clinical practice. Flare of an underlying autoimmune condition and development of new immune toxicity in these patients have been reported in many case series[13, 22]. Whether ICI leads to progression of autoimmune disease in patients with existing subclinical autoimmune disease is not fully understood. De Moel and colleagues hypothesised that the non-specific increase in B cell activation and autoantibody production induced by ICI may be behind many irAEs. They measured 23 common autoantibodies including those associated with connective tissue disease, autoimmune liver disease, thyroiditis and coeliac disease before and 12 weeks after ipilimumab in 133 melanoma patients. 20% of the cohort were positive for any antibody at baseline, and 29% were antibody positive after treatment. Baseline autoantibodies were not associated with irAEs however patients with post-treatment antibodies were more likely to develop irAEs. Of all autoantibodies, the strongest association was between new seropositivity to thyroid autoantibodies and thyroiditis (odds ratio [OR] 6.26, 95% CI 1.07-36.5; p0.04) [26] The small total number of irAEs occurring in this cohort limited the power of the study to demonstrate significant associations between other autoantibodies and their associated organ toxicity.

Genetic predisposition in the form of certain Human Leukocyte Antigen (HLA) class I and II

alleles are well documented in spontaneous autoimmune disease including rheumatoid arthritis, Behcet's disease, ankylosing spondylosis and type 1 diabetes[27]. These associations also appear to apply to irAEs. High risk class II HLA haplotypes associated with rheumatoid arthritis and type 1 diabetes have been found in patients with ICI related inflammatory arthritis and diabetes respectively [28, 29].

Treatment of irAEs

For most irAEs, Grade 3-4 symptoms are the threshold to withhold ICI and treat with oral or systemic glucocorticoids. A tissue biopsy may be performed to exclude alternate pathology such as infection in the case of colitis and pneumonitis. In Grade 4 (life threatening) toxicity, the patient is admitted to hospital and generally receives pulsed intravenous methylprednisolone, often followed by a course of oral glucocorticoids that is progressively reduced over time. Steroid-sparing agents are utilised for specific indications such as the TNF alpha inhibitor infliximab (colitis, pneumonitis, arthritis), the integrin alpha4beta7 inhibitor, vedolizumab (colitis), mycophenolate mofetil (hepatitis, pneumonitis) and anti-thymocyte globulin (colitis, hepatitis)[30-32]. While high dose glucocorticoids are still recommended in patients with Grade 4 endocrine toxicity, this has not been prospectively evaluated in clinical trials and at present, no evidence of reversal of endocrine hormone production has been demonstrated. As endocrine gland toxicity tends to result in permanent loss of gland function, withholding ICI after onset of overt endocrine dysfunction may be of no benefit.

The remainder of this literature review will outline the incidence, mechanisms, natural history and specific management of endocrine irAEs.

1.3 Diabetes associated with Immune-Checkpoint Inhibition

Type 1 diabetes is caused by self-reactive T cell-mediated islet destruction, resulting in a permanent inability to secrete insulin and a lifelong dependence on exogenous insulin. Diabetes after ICI appears to be limited to inhibition of the PD-1/PD-L1 pathway, with no definite cases associated with CTLA-4 inhibition[29]. The relationship between the PD-1/PD-L1 pathway and diabetes has been modelled in many pre-clinical mouse studies. PD-1/PD-L1 knock out non-obese diabetic (NOD) mice develop insulitis and diabetes much sooner than wildtype mice. Similarly, the injection of anti-PD-1 or anti-PD-L1 rapidly induces diabetes in NOD mice, while administration of anti-CTLA-4 has no impact on progression to diabetes. The histological finding that PD-L1 is upregulated on pancreatic islets in NOD mice indicates a last-line protective mechanism against autoimmunity[33]. It is likely that the islet-reactive T cell response is being kept at least partly "in-check" by PD-1/PD-L1 negative co-stimulation prior to overt diabetes onset which is why anti PD-1/PD-L1 agents trigger onset.

Clinical phenotype, diagnostic features and natural history

In early clinical trials between 2012-2015, only two episodes of diabetes associated with inhibition of the PD-1/PD-L1 pathway were reported, with little or no details provided about the presentation or biochemistry [34, 35]. In 2015, the first direct evidence of an autoimmune mechanism behind new onset diabetes was reported by Herold and colleagues in a case series of 5 patients presenting with severe hyperglycaemia or diabetic ketoacidosis (DKA) after PD-1 inhibition[36]. High titres of islet autoantibodies were found at presentation in three of the five patients, high-risk class II HLA alleles in four, and increased islet antigen specific $CD8^+$ T cells in two patients. Thus, the authors concluded there was evidence of both cellular and humoral immune responses to self-antigen and termed this entity "checkpoint inhibitor related autoimmune diabetes mellitus (CPI-DM)." Multiple case reports followed, including

the case series and literature review included in Chapter 2 (Publication 1) of this thesis, which now suggest an incidence approaching 1%[37]. A systematic review published in 2019 found 71 published reports. Consistent with Publication 1, the authors described a phenotype of accelerated type 1 diabetes[29]. 71% of all cases occurred in the first 3 months after treatment with a PD-1 inhibitor. This review pointed out important similarities and differences between CPI-DM and spontaneous onset type 1 diabetes. Firstly, complete loss of islet function at diagnosis was manifest by a low or undetectable c-peptide (a marker of endogenous insulin production) in all cases. A relatively low HbA1c at presentation suggested that the islet destruction occurred over a short period of time. Autoantibodies to glutamic acid decarboxylase (GAD) were found in 50% of cases at onset of CPI-DM, and people with elevated antibodies were more likely to present early and with DKA. Class II HLA genes known to infer increased risk of spontaneous diabetes (DR4-DQ8, DR3-DQ2) were found in 85% of patients. A rise in the pancreatic enzymes amylase and lipase have been reported, on some occasions in parallel with significant loss of pancreatic volume when serial imaging has been performed[33]. I explore these changes further in Chapter 2.3.

In contrast to CPI-DM, spontaneously occurring type 1 diabetes develops over many months to years, beginning with a pre-clinical phase (now called stage 1 T1D) when evidence of autoimmunity may be present before onset of dysglycaemia (stage 2) and overt diabetes (stage 3)[38]. At presentation, the HbA1c is generally higher, suggestive of a longer sub-acute phase of evolving hyperglycaemia[33]. At diabetes onset, >90% of individuals with spontaneous type 1 diabetes will have increased titres of GAD autoantibodies (nearly double the frequency seen in CPI-DM). The frequency of high-risk genes within the HLA region is very similar between CPI-DM and spontaneous type 1 diabetes. Like in spontaneous type 1 diabetes, patients with CPI-DM are left permanently insulin dependent and prone to diabetic

ketoacidosis[29]. A second important comparison is that between CPI-DM and the subtype of idiopathic diabetes termed “Fulminant Diabetes.” Observation of patients who lack typical autoantibodies or evidence of insulitis on pancreatic biopsy at the time of beta cell destruction previously lead to the classification of Type 1 diabetes into immune mediated (type 1A) and idiopathic (type 1B)[39]. In 2000, Imagawa and colleagues reviewed consecutive patients presenting with type 1 diabetes and further described a subset of patients presenting with abrupt onset beta cell destruction and ketoacidosis who lacked diabetes autoantibodies. These patients presented with near normal HbA1c levels and a mean duration of hyperosmolar symptoms of 4 days. C peptide levels were very low or absent. Evidence of generalised pancreatic inflammation in the form of markedly elevated serum pancreatic enzyme levels and lymphocytic infiltration of the exocrine pancreas on biopsy differentiated these patients from those with the more classic type 1A presentation. The authors suggest that the combination of rapid onset disease, lack of antibodies and generalised pancreatic inflammation should be classified as “Fulminant Diabetes”[40]. Whether an alternate mechanism (such as a viral initiated beta cell inflammation) exists in this subset or that the underlying autoimmune response has simply not been detected by standard laboratory techniques is still not known.

Fulminant diabetes is now thought to account for up to 20% of type 1 diabetes presentations in Japan[41]. Although histopathological analysis is rarely performed in patients presenting with CPI-DM, patients presenting with diabetes after PD-1 inhibitors frequently share this phenotype. In light of these similarities, Baden and colleagues sought to evaluate whether such a classification system may differentiate patients presenting with beta cell failure after PD-1 inhibition. The authors contacted members of the Japan Diabetes Society (JDS) as well as authors of case reports with a questionnaire regarding demographic, clinical and

immunological features of patients diagnosed with diabetes after PD-1 inhibition. Two distinct phenotypes were defined for the purpose of sub-classifying cases of anti-PD-1/PD-L1 diabetes. The first, termed “acute onset type 1 diabetes” was defined by the JDS as a presentation with ketosis or ketoacidosis and low c peptide requiring permanent insulin treatment within 3 months of hyperglycaemic symptom onset with or without detectable islet autoantibodies. “Fulminant diabetes” was then defined as a presentation with ketosis or ketoacidosis within 1 week of hyperglycaemic symptom onset with a low c peptide and HbA1c below 8.7% at the first visit. Pancreatic enzyme elevation was not a diagnostic criterion. In a cohort of 22 patients, retrospective analysis of information obtained through the clinician surveys demonstrated 50% of patients met criteria for “fulminant diabetes.” Within the constraints of a very small sample size, the authors concluded that patients meeting criteria for fulminant diabetes were more likely to present with systemic symptoms and had a lower mean arterial pH at diagnosis[41]. This study raises the interesting question of whether two distinct clinical phenotypes within patients presenting with CPI-DM may reflect differential underlying mechanisms, however major limitations prevent accurate mechanistic insights. The first relates to the small sample size of 22 patients obtained from retrospective physician surveys across multiple sites with no known denominator. The authors were unable to demonstrate a statistically significant difference in any clinical or biochemical features between the groups. The rationale for selected criteria for the two clinical phenotypes is not detailed, and presumably does not account for some overlap syndrome in patients presenting early but after one week of hyperglycaemic symptoms. Finally, performing the study in Japan is of relevance to local clinicians where the incidence of spontaneous fulminant diabetes is higher, but whether the results will extrapolate to patients of alternate ethnic background is not known.

Knowledge gaps relating to CPI-DM

Several notable gaps in the literature exist in the understanding of CPI-DM. The first relates to the role of baseline islet autoantibodies and HLA status in predicting the risk of developing CPI-DM, which are yet to be prospectively evaluated.

About 4% of the healthy adult population have recordable islet antibodies (predominantly GAD antibody)[42]. In the absence of checkpoint inhibition, cohort studies suggest that around 10% of subjects with a single islet autoantibody will progress to diabetes[43, 44]. Inhibitory checkpoint blockade may cause more antibody positive subjects to develop diabetes via removal of restraining mechanisms. When exposed to PD-1 inhibition, a T cell response is seen in most subjects however only 20-30% will demonstrate tumour regression[34, 45]. If 20% of subjects with a positive GAD antibody progress, about 1% of the adult population receiving check point inhibitors will be susceptible to diabetes, in line with the recently reported incidence of CPI-DM[29]. An alternate consideration is that that progression to autoimmune diabetes occurs without autoantibodies in some cases. I evaluate this relationship retrospectively in Chapter 2 and in the design of a prospective study summarised in Chapter 5.

The second gap relates to the potential for early reversal of CPI-DM with immunomodulation. High-dose glucocorticoids, the TNF-alpha inhibitor infliximab and other biologics have been utilised with success in the treatment of non-endocrine irAEs such as colitis, hepatitis, pneumonitis and arthritis[30]. Phase II trials in children and young adults with new onset type 1 diabetes have suggested potential benefit of the anti-CD3 antibody teplizumab and more recently the TNF alpha inhibitor Golimumab in delaying islet failure but to date, there are no agents licenced for the reversal of type 1 diabetes or CPI-DM[46, 47]. The treatment effect of high-dose glucocorticoids in CPI-DM has been evaluated in

single case reports only, with no prospective, randomised treatment trials undertaken [37, 48]. In NOD mice, induced TNF receptor deficiency infers complete protection from diabetes[49]. One case of reversal of hyperglycaemia after ICI with infliximab was reported by Trinh and colleagues. We review this paper and respond in a letter to the editor in Chapter 2.4.

1.4 Hypophysitis associated with Immune-Checkpoint Inhibition

Historically, autoimmune hypophysitis was a rare diagnosis occurring in only 1 in 7-9 million people, and usually made after exclusion of other causes of hypopituitarism and/or pituitary enlargement such as apoplexy or macroadenomas[50]. Spontaneous lymphocytic hypophysitis has a 9:1 female predominance and can be associated with marked pituitary enlargement in the setting of prolonged T cell infiltration prior to symptom onset. The clinical syndrome may be a combination of compressive symptoms like headache and visual changes or may relate to pituitary hormone dysfunction[51].

Clinical phenotype, diagnostic features and natural history

Approval and widespread use of immune checkpoint inhibitors has resulted in a dramatic increase in the incidence, with up to 15% of patients receiving anti-CTLA-4 developing hypophysitis[50, 52, 53]. Hypophysitis due to treatment with anti-PD-1/PDL-1 monotherapy remains rare at 0.4-0.7%[54]. In the landmark CheckMate 067 melanoma trial, hypophysitis occurred in the 7% of patients in the ipilimumab and nivolumab combination arm, <1% in the nivolumab arm and 4% in the ipilimumab arm, suggesting that anti-CTLA-4 therapy is the major driver of this immune-related adverse event (irAE)[9, 55].

In a review of 71 cases of hypophysitis, fatigue and headache were the most common symptoms occurring in 59% and 61% respectively. Anterior pituitary hormone dysfunction in the adrenal, thyroid and gonadal axes occurred in 96%, 91% and 73% respectively[56]. In contrast to spontaneous hypophysitis, a gender predominance has not emerged. The overall increase in gland size does not appear to be as marked as in spontaneous hypophysitis, which may reflect the shorter time frame over which the pituitary inflammation occurs.

The mechanisms behind CTLA-4 induced hypophysitis are not completely understood. In 2014, Iwama and colleagues hypothesised that CTLA-4 is expressed on pituitary endocrine cells and were able to demonstrate this with murine and human models. CTLA-4 messenger ribonucleic acid (mRNA) was detected on pituitary cells, while CTLA-4 was not expressed in brain, thyroid or adrenal tissue. The authors then demonstrated seroconversion of pituitary antibodies against prolactin and adrenocorticotropic hormone (ACTH) secreting cells in mice injected with ipilimumab, with no seroconversion in the control group who received hampster IgG. Finally, they measured pituitary antibodies in 20 human subjects before and after administration of ipilimumab. In the 7/20 patients with diagnosed hypophysitis, new pituitary antibodies developed after ipilimumab while no antibodies developed in the other 13 patients who received ipilimumab but did not develop hypophysitis[57]. At present, a pituitary antibody assay is not commercially available, and these results are yet to be replicated.

Knowledge gaps relating to checkpoint inhibitor induced hypophysitis

At the time of this literature review, international guidelines including the European Society of Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO) and the Society for Immunotherapy of Cancer (SITC) suggest evaluating for pituitary hormone deficiency after ICI only when clinical suspicion for hypophysitis emerges[30-32]. Based on

the published case series, the clinical presentation can be mild and non-specific. We hypothesised that the true incidence of hypophysitis may be underappreciated without regular biochemical screening and careful review of routine brain imaging performed during the course of treatment with ICI. This hypothesis is studied and discussed in Chapter 4.

The development of a commercially available pituitary antibody assay would allow for greater insights into the humoral response after CTLA-4 inhibition. Plans for the development of this novel assay are summarised in Chapter 5, Future Directions.

1.5 Thyroiditis associated with Immune-Checkpoint Inhibition

In understanding the natural history of ICI related thyroiditis, it is useful to compare and contrast this entity to spontaneous autoimmune thyroid disease. In the general endocrine population, Graves' disease is the most common cause of hyperthyroidism. This form of autoimmune thyroid disease is driven by stimulating (or more rarely, blocking) autoantibodies to the thyroid stimulating hormone receptor (TSHrAb)[58]. Graves' disease typically causes a prolonged state of thyroid hormone over-production, which can be demonstrated by diffusely increased uptake on a technetium (Tc99m) scan. Patients generally require 6-18 months of treatment with antithyroid drugs or can be definitively managed with thyroidectomy or radioactive iodine ablation[59]. Hashimoto's disease or chronic autoimmune thyroiditis are the most common causes of hypothyroidism in iodine sufficient areas. In the presence of high levels of antibodies against the thyroid antigens thyroid peroxidase (TPOAb) and thyroglobulin (TgAb), diffuse T cell and B cell infiltration into the gland leads to thyroid epithelial cell apoptosis and gradual gland failure over months to years[60]. In Hashimoto's disease and other forms of inflammatory thyroiditis (post-viral or post-partum) an early, transient thyrotoxic phase may occur due to release of pre-formed

thyroid hormone after thyroid inflammation. A Tc99m scan differentiates these pathologies from a state of thyroid hormone overproduction by the presence of diffusely low uptake throughout the gland.

Clinical phenotype, diagnostic features and natural history of ICI induced thyroiditis

Thyroiditis is one of the most common irAEs after ICI[13]. In the landmark Checkmate 067 trial comparing combination ipilimumab and nivolumab to single agent ipilimumab and single agent nivolumab, thyroiditis occurred in 28%, 6% and 15% respectively[9]. Unique to other forms of spontaneous thyroid autoimmunity, ICI appears to induce a rapidly destructive, inflammatory thyroiditis[61]. Although clinical trials and case series report a range of thyroid problems after ICI including subclinical hyperthyroidism, overt thyrotoxicosis and hypothyroidism, these biochemical states most probably reflect time points on the same disease trajectory. When thyroid function tests are performed routinely and frequently, an early thyrotoxic phase usually precedes a hypothyroid phase which is permanent at least 50% of the time[62].

The role of thyroid autoantibodies in ICI related thyroiditis is less clear, with 45% and 33% of patients found to have elevations in antibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TGAb) respectively[62], compared to 95% and 80% in spontaneous autoimmune thyroid disease[63]. New onset Graves' disease after ICI is extremely rare but has been described in case reports, and guidelines do recommend measuring TSHrAb in any case of severe or symptomatic thyrotoxicosis to guide initiation of anti-thyroid drugs[30].

In case reports where a technetium 99m (Tc99m) scan was performed, diffusely low uptake in the thyroid was observed during the thyrotoxic phase, suggesting that like in other forms of

thyroiditis, the transient thyrotoxic phase relates to release of preformed thyroid hormone into the circulation during thyroid inflammation[64]. In contrast to spontaneously occurring thyroiditis, the thyrotoxic phase after immune checkpoint inhibition is very short[62]. Tc99m is rarely performed and not recommended in international guidelines for the management of irAEs[31].

Knowledge gaps relating to checkpoint inhibitor induced thyroiditis

The role of antibodies in ICI related thyroiditis remains unclear despite having been evaluated in several prospective studies between 2017-2019. The lower prevalence of antibodies after ICI related thyroiditis may be due to rapid onset T-cell mediated autoimmunity causing disease prior to formation of antibodies in some patients. Alternatively, it is possible that novel autoantibodies are formed which are not detected in the current assay. It appears that the presence of thyroid autoantibodies at baseline may infer increased risk of ICI related thyroiditis, but existing studies have significant limitations. Kobayashi and colleagues measured TPOAb, TgAb and TSHrAb at baseline and every 6 weeks after treatment with single agent nivolumab in 66 patients with a variety of primary malignancies. Patients who developed thyroiditis were significantly more likely to have had elevated titres of TPOAb and TGAb at baseline. The sample size of 66 patients was the major limitation of this study. 3/6 patients with existing antibodies developed thyroiditis. Of the 60 patients with negative baseline antibodies, 5 developed new antibody seroconversion but only one developed thyroiditis[65]. Similar results were produced by Osorio and colleagues in another small prospective study of 51 patients treated with pembrolizumab[66]. Yukihiro and colleagues performed a larger but retrospective study in 137 patients treated with nivolumab or pembrolizumab who had stored biospecimens at baseline and after treatment. Rheumatoid factor (RF), antinuclear antibody (ANA), TPOAb and TgAb were measured on stored

specimens. The authors drew several conclusions. First, the presence of existing thyroid antibodies prior to treatment was associated with a higher incidence of thyroiditis (20% versus 1%, $p<0.01$). Secondly, the presence of any of the measured antibodies was associated with both improved tumour outcomes and a higher incidence of irAEs, however this relationship was not significant for thyroid antibodies alone. [67]. Overall, available literature suggests that it is unlikely that antibodies are the main mechanism of disease in ICI related thyroiditis. Rather, a pathogenic T cell response may be accompanied by an antibody response, the main significance of which is as biomarkers. The B lymphocytes producing anti-TPO and anti-TG antibodies are likely to be involved as antigen presenting cells.

As one of the more common irAEs, the question of whether thyroiditis may correlate with favourable tumour outcomes is of great relevance. In January 2020, Kotwal and colleagues published a retrospective study in 91 patients treated with the PD-L1 inhibitor atezolizumab. Thyroiditis occurred in 25% of the patients. While median overall survival cut off was not reached in the thyroiditis group, overall mortality was significantly lower compared to patients without thyroiditis (44% versus 79% $p=0.001$)[68]. The authors adjusted for age, sex and cancer type but importantly not for tumour burden, functional status or treatment duration. Overall, small sample size and confounding variables have limited many studies examining this relationship.

1.6 Rare endocrinopathies associated with immune-checkpoint inhibition

Autoimmune Adrenalitis

Primary adrenalitis was very rare in phase III clinical studies[69]. Biochemical data provided in case reports, however, does support the existence of this irAE. Unlike the more non-specific presentation of secondary adrenal insufficiency due to hypophysitis[50], these

patients present in adrenal crisis with hypotension, hyperkalaemia and hyponatraemia. The presence of an elevated ACTH distinguishes primary from secondary adrenal failure. Adrenal cortex and 21-hydroxylase antibodies have been recorded and imaging demonstrates interval adrenal gland atrophy[70].

Autoimmune Hypoparathyroidism

Acute, symptomatic hypocalcaemia attributed to immune hypoparathyroidism after ICI has now been reported in at least 5 case reports[71]. Antibodies to the calcium sensing receptor are detected in up to 50% of patients with spontaneous autoimmune hypoparathyroidism[72] and have now been reported in two patients with ICI related hypoparathyroidism[71]. NALP5-specific autoantibodies have been detected in patients with autoimmune polyglandular syndrome type 1 and hypoparathyroidism but are not yet reported after ICI[73].

1.7 The scope of this thesis

At the beginning of this project, many of the mechanistic and clinical insights highlighted in Chapter 1 were not known, and to date several knowledge gaps still exist. This thesis aims to explore clinical, immunological and radiological aspects of the three most common ICI related endocrinopathies: diabetes, hypophysitis and thyroiditis in a local cohort. As the use of combination ICI increases within multiple tumour streams, developing an understanding of these factors will be critical in the pre-treatment counselling and in the day-to-day management of patients treated with ICI.

A large component of each chapter focuses on the findings from a retrospective audit of patients treated with combination ICI at a melanoma referral centre. A detailed description of the aims and methods of this study are outlined in Chapter 1.8.

1.7.1 Chapter 2: PD1 inhibitor related autoimmune diabetes and pancreatitis

When I embarked on this research, the understanding of ICI induced diabetes was based on emerging case reports of patients who presented with diabetic ketoacidosis (DKA) after treatment with the anti-PD-1 agents pembrolizumab or nivolumab[36]. By 2018, there were 28 cases of this phenomenon reported world-wide, with limited Australian data. Publication 1 is a review of the published case reports, and a case series of 9 patients with anti-PD-1 related diabetes diagnosed in Melbourne, Australia.

I describe an accelerated, fulminant form of islet autoimmunity which manifests as DKA or symptomatic hyperglycaemia requiring hospital admission soon after the first treatment with anti-PD-1. The discussion draws a parallel to the fulminant diabetes previously reported in Asian countries as well as Australia[74]. I specifically evaluated the available data on HLA and islet autoantibodies as predictive biomarkers.

I then set out to describe the local incidence of diabetes and pancreatitis after combination immune checkpoint inhibition with a retrospective audit. While no cases of classic CPI-DM were identified, changes in pancreatic enzymes and in pancreatic imaging are discussed, raising the potential for alternate phenotypes of pancreatic irAEs.

During the retrospective study and in my role as endocrine research fellow at the VCCC, I began to see cases of diabetes and pancreatitis after anti-PD-1 which were atypical compared

to the acute form described in publication 1. I presented an overview of classic and non-classic diabetes and was a finalist in the poster presentation awards at the Australian Diabetes Congress in 2019 (Appendix 1). I was then involved in the preparation of a manuscript describing two patients with CPI-DM whose presentation had been masked by high dose glucocorticoids (Appendix 2). I summarise these findings and discuss how to identify CPI-DM in cases that present atypically.

In July 2019, a case was published in the high impact journal Diabetes Care, suggesting a potential therapeutic role of the tumor necrosis factor (TNF)-alpha inhibitor Infliximab in reversing islet autoimmunity after ICI[75]. In light of my exploration into classic and non-classic forms of anti-PD-1 related diabetes, I was not convinced that the case in question reflected autoimmune diabetes. Rather, that the hyperglycaemia may have reflected insulin resistance induced by intra-articular steroid injection. I conclude this chapter with Publication 2; outlining my reflections on this case in a "Letter to the editor".

1.7.2 Chapter 3: Hypophysitis in patients treated with combination ICI

Unlike autoimmune diabetes, hypophysitis is most commonly reported after treatment with anti-CTLA-4. Whether the addition of an anti-PD-1 agent in combination therapy increases the risk is not clear. What was striking when reading international guidelines and published case series, was the lack of recommendations regarding routine monitoring of pituitary hormones or hormones dependent on the pituitary in patients undergoing treatment with ICI. In light of the known non-specific nature of the hypophysitis presentation, I hypothesised that the true incidence may be underappreciated. Chapter 3.1 is a manuscript prepared for submission and pending peer review titled "The complementary role of routine cancer imaging in identifying clinically-occult hypophysitis after combination immune checkpoint inhibition." The aims of this study are to;

1. Describe the prevalence and clinical features of patients with documented hypophysitis following treatment with combination ICI in a retrospective cohort of 162 patients with advanced melanoma who had relatively frequent monitoring of cortisol and thyroid function;
2. Systematically assess the utility of routinely performed neuroimaging and FDG-PET in the detection of hypophysitis;
3. Evaluate inter-observer agreement of FDG-PET as a novel modality for the detection of hypophysitis;
4. Correlate the presence of hypophysitis with treatment outcome

This was the first large observational study specifically investigating the incidence and diagnostic features of hypophysitis in patients treated with combination CTLA-4 and anti-PD-1.

In collaboration with co-authors from the Department of Cancer Imaging, I was then involved in the production of a pictorial essay highlighting the classic imaging features of hypophysitis and how to distinguish these from alternate pathologies such as macroadenomas and pituitary metastases (Appendix 3.) I summarise these findings and conclude the chapter with key messages relating to the diagnosis of immune checkpoint inhibitor related hypophysitis.

1.7.3 Chapter 4: Thyroiditis in patients treated with combination ICI

Thyroiditis is a common endocrine irAE after both anti-CTLA-4 and anti-PD-1 treatment. At our local centre, many clinicians were utilising antithyroid drugs for more severe onset

thyrotoxicosis while awaiting the results of the follow up thyroid function tests and autoantibodies. Based on the outcomes from my literature review, I hypothesised that ICI induced thyroiditis was predominantly a destructive phenomenon not associated with autonomous overproduction of thyroid hormone and set out to map the natural history of this entity in the local cohort. In the absence of routine use of Tc99m scans in the oncology cohort, I then hypothesised that increased thyroid uptake on FDG-PET imaging (seen incidentally with increasing frequency on tumour surveillance studies) may correlate with ICI induced destructive thyroiditis. Chapter 4 is a manuscript prepared for publication and pending peer review. The aims of Chapter 4 are to;

1. Describe the incidence and natural history of thyroiditis in our retrospective cohort of 162 patients with advanced melanoma;
2. Review the presence of thyroid autoantibodies at presentation
3. Evaluate the diagnostic accuracy of routine FDG-PET in ICI related thyroiditis according to the STARD 2015 reporting standards
4. Evaluate the interobserver agreement of FDG-PET in the detection of thyroiditis

This study adds to the published literature defining ICI related thyroiditis as a destructive phenomenon and is the first to describe the diagnostic accuracy of FDG-PET in thyroiditis.

1.7.3 Chapter 5: Summary, conclusions and future directions

The contents of this thesis are linked together with a summary of the key findings and overall conclusions, followed by an outline of the future directions. The latter includes the design of a study evaluating the presence of thyroid and islet autoantibodies at baseline and after ICI

and HLA in a local cohort, as well as the development of a novel pituitary antibody assay aiming to further address knowledge gaps and improve patient care.

References are presented at the end of this thesis followed by three appendices.

1.8 Methodology

A significant component of each chapter is the analysis of data obtained in a retrospective study performed at Peter MacCallum Cancer centre in collaboration with medical oncology, nuclear medicine and cancer imaging. A description of the specific aims and methods relating to each endocrine toxicity are outlined in the respective chapters. An overview of the background, aims and methods for the study as a whole is presented in this chapter.

Background

Endocrine toxicities occur with differential frequencies depending on the type of immune checkpoint inhibition. For example, hypophysitis is seen predominantly with CTLA-4 inhibition, diabetes is most common after inhibition of the PD-1/PD-L1 pathway and thyroiditis may occur with either but most commonly with PD-L1 inhibition[69]. In general, irAEs occur more often and with increased severity in patients treated with combination immune checkpoint inhibition (cICI)[9], however little detail regarding specific endocrine toxicity was provided in stage III clinical trials comparing cICI to anti-CTLA-4 and anti-PD-1 monotherapy. cICI is now standard therapy for the treatment of advanced melanoma in our centre, however the local incidence of endocrine toxicity after cICI has not been evaluated and the role of diagnostic tools such as routine biochemistry and imaging are not well defined.

Research Questions

1. What is the incidence of diabetes, hypophysitis and thyroiditis after cICI?
2. What is timing of onset and natural history of these endocrine toxicities?
3. What is the diagnostic accuracy of FDG-PET/CT in the identification of diabetes, hypophysitis and thyroiditis?

4. What are the defining radiological features of cICI related hypophysitis as compared to other pituitary pathologies?

Aim

To describe the incidence, natural history and diagnostic features of endocrine toxicity in patients treated with combination immune checkpoint inhibition (cICI)

Methods

Consent Procedure

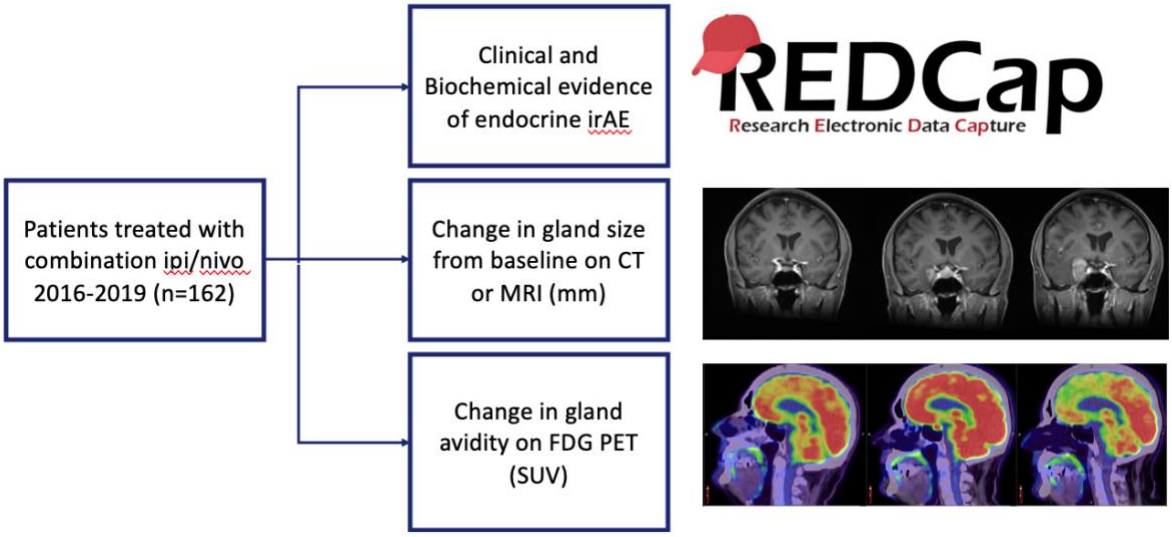
A waver of consent has been approved by the Victorian Comprehensive Cancer Centre (VCCC) Human Research Ethics committee (HREC 17/231). Individual patient consent was not required as no patient was contacted or impacted by this retrospective audit. No identifying information will be passed on to any third party.

Development of a data collection tool

I designed a data dictionary to collect relevant patient, tumour, treatment toxicity and response variables. This tool was presented to a focus group of medical oncologists who provided feedback on the oncologic variables. Important toxicity variables included organ system, grade, time to onset from commencing cICI, the number of cycles administered prior to onset, relevant biochemistry and treatment information. I met with a radiologist and a nuclear medicine physician within the Department of Cancer Imaging to discuss the imaging features of endocrine irAEs. These specialists confirmed the lack of recognised criteria for the radiological diagnoses, but provided clinical expertise into the relevant radiological variables to collect including but not limited to cranio-caudal pituitary size measured in the sagittal plane on MRI and standardized uptake value (SUVmax) generated by FDG-PET/CT.

I built these variables into a set of baseline and repeating instances within the secure REDCap data storage platform, to enable tracking of the timing of toxicities as well as biochemical and radiological diagnostic tests with respect to the treatment commencement date (Figure 1).

Figure 1. Methodology schematic



Clinical and biochemical data

I obtained a pharmacy dispensing data report to identify patients who have received at least one dose of any immune checkpoint inhibitor between January 2016 – Jan 2019. Using the pivot table function in excel, I selected patients who had received cICI and uploaded the record ID, date of birth and prior treatment data directly to REDCap. Patient demographics and relevant clinical variables were collected from the institutional online medical record (Clinical Viewer) and entered manually into REDCap.

A pathology extract was provided which included cortisol and thyroid function test results for all patients presenting to the in-house pathology service within the specified time frame. The V-Look up function was utilized on excel to identify any results relevant to the selected cohort. Additional pathology results were obtained from Clinical Viewer, with a particular

focus on pituitary and thyroid hormones, thyroid and islet antibodies, lipase, glucose and HbA1c.

An oncology fellow was responsible for the entry of tumour and response variables, which were not reported in detail in this thesis but will contribute to ongoing collaborative projects.

Radiological data

A single neuroradiologist, blinded to clinical outcomes reviewed all CT and MRI imaging covering the pituitary gland at baseline and during the first 6 months of cICI treatment. Pituitary size and appearance before and after treatment were entered by the radiologist into the REDCap database. Imaging criteria for cICI-induced hypophysitis have not been established. Based on the clinical experience of the radiologist, we defined the MRI imaging criteria for imaging detected hypophysitis as a relative change in pituitary gland size by ≥3mm after cICI.

In parallel, serial FDG-PET/CT scans were assessed by a nuclear medicine physician (NMP) blinded to the clinical data. In the absence of established qualitative or semiquantitative criteria suggestive of hypophysitis, thyroiditis or pancreatitis on FDG PET/CT, for this study, a visibly perceptible increase in FDG uptake from baseline in one of these glands was considered abnormal. Given the subjective nature of this assessment, an interobserver agreement was built into the study design, with a second NMP independently reviewing all images. Then, an exploratory semiquantitative analysis was performed using a $1cm^2$ spherical region of interest over the pituitary, thyroid and pancreas to assess maximum standardised uptake value (SUVmax) at baseline, post-treatment initiation and until resolution.

The specific methodology, statistical analyses, results and conclusions for each toxicity are outlined in their respective chapters.

Chapter 2. Immune checkpoint inhibitor related diabetes and pancreatitis

2.1 Introduction

Checkpoint inhibitor related autoimmune diabetes mellitus (CPI-DM) was rarely reported in clinical trials, however, became increasingly recognised and studied during the time of my candidature. Chapter 2 provides a chronological summary of my findings with regards to CPI-DM, beginning with a publication titled "Diabetes associated with immune check point inhibition – presentation and management challenges." This publication includes a detailed analysis of 9 local cases and a summary of the published literature at the time. I then present the findings relating to diabetes and pancreatitis from the retrospective study outlined in Chapter 1.8. These findings prompted an exploration into non-classic forms of diabetes after ICI in the form of a case-based discussion. Finally, I conclude this chapter with a discussion of the role of immunomodulation in reversal of diabetes, presented in a "letter to the editor" publication.

2.2 Publication 1: A case series and literature review

Diabetes associated with immune check point inhibition – presentation and management challenges

Diabet. Med. 35, 1283–1290 (2018) DOI: 10.1111/dme.13762

Diabetes associated with immune check point inhibition – presentation and management challenges.

Short running title: Immunotherapy related type 1 diabetes – a case series.

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A bulleted novelty statement:

- Immune checkpoint inhibitor related type 1 diabetes was rare in clinical trials, however case reports between 2015-2017 suggest that real-world incidence may be higher than previously anticipated.
- We describe 9 cases of this life threatening immune-mediated type 1 diabetes. To our knowledge this is the largest series reported in the literature to date.
- Patients presented with fulminant, symptomatic hyperglycaemia within weeks of commencing immune checkpoint inhibitors.
- The unprecedented survival benefit seen with immune checkpoint inhibitors across many cancers is anticipated to result in widespread use as single agent and in combination with other cancer therapies in the near future.

Abstract

Background: In recent years, immune checkpoint blockade has become a standard therapy for a wide range of cancers. Adverse events including endocrinopathies result from the induction of autoimmunity.

Case report: We report a case series of nine individuals who presented with immunotherapy-induced type 1 diabetes between 2015-2017. Onset of diabetes occurred within 12 weeks of commencing therapy. Anti- GAD antibodies were present in six people. Retrospective testing of islet antibodies in pre-treatment samples was possible in two people and this revealed anti-GAD seroconversion in the first and high anti-GAD titres pre and post-treatment in the second person. Six people had high risk HLA haplotypes. Clinical and genetic factors are described and compared with previously published cases.

Conclusion: This rare form of iatrogenic diabetes is a result of an accelerated, fulminant islet autoimmunity induced by immune checkpoint inhibitors. High risk human leukocyte antigen haplotypes have been found in the majority of cases. The role of pre-existing islet autoantibodies as a biomarker is not yet known.

Key words

Type 1 diabetes, Immunotherapy, Immune check-point inhibitor related diabetes, immune-related adverse event, immunology.

Introduction

Immunotherapy is based on the premise that an enhanced immune system can recognise and eradicate cancers. Checkpoint blockade with cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) and programmed cell death protein-1 pathway (PD-1/PD-L1) is rapidly becoming standard care in a range of cancers including melanoma, non small cell lung cancer, renal cell

carcinoma and head and neck cancer with unprecedented impact on overall survival (1-5).

CTLA-4 and PD1 are inhibitory cell-surface receptors expressed on activated T cells that limit immune activation. In contrast to anti-CTLA-4 that amplifies the T cell activation in lymphoid organs and tumours, anti-PD1 predominantly overcomes exhaustion of anti-tumour T effector cells within the tumour bed. T cell exhaustion represents a state of cellular dysfunction induced during chronic antigen/tumour exposure, leading to failure to control tumour progression (6). A common analogy is that checkpoint inhibitors act by "releasing the brakes" on the immune system to combat the tumour (7). Conversely, T cell exhaustion phenotypes are thought to infer better prognosis in autoimmune diseases. With restoration of exhausted T cell function mediated by PD-1 pathway blockade, induction of collateral immune-related adverse events (irAEs) such as rash, hepatitis, nephritis, pneumonitis, colitis and endocrinopathies are common. While most irAEs are responsive to immunosuppression, endocrinopathies tend to be irreversible and require ongoing hormone replacement (8).

The incidence of immunotherapy induced type 1 diabetes is reported to be approximately 0.4% (1). However, reports suggest that the real-world incidence may be significantly higher (8-32). Cohort studies of islet antibody positive people (mainly first-degree relatives of people with type 1 diabetes) have found that approximately 10% with a single positive antibody will progress to type 1 diabetes (33). Factors that lead to progression to diabetes may include differences in gene expression of diabetogenic T cells, viral antigen reactivity and metabolic pathways used by autoreactive T cells (34). The relationship between PD-1 and its ligands in the development of diabetes has been established in mice. PD-1–PD-L1 interaction inhibits the activation, expansion, and effector function of islet-reactive T cells through the life span of the animal. (35).

In keeping with the mechanism of PD-1 pathway blockade, one hypothesis is that progression to fulminant islet autoimmunity may be increased or accelerated in a proportion of healthy individuals with positive islet antibodies.

Research Design and Methods

We describe our experience of nine people presenting with anti-PD1 mediated type 1 diabetes between 2015-2017. Treatment was undertaken at two tertiary centres in Melbourne, Australia: The Peter MacCallum Cancer Centre and Eastern Health. The authors were responsible for management and follow up. Demographics, treatment history, diabetes presentation, the presence of islet antibodies and HLA type were characterised. Where feasible serum obtained pre-treatment was tested for evidence of pre-existing islet autoimmunity.

A literature review of PubMed and Medline for case reports of anti-PD1 related diabetes identified 28 cases. Clinical information from our series was compared with published cases, with a focus on timing and kinetics of diabetes in relation to commencing anti-PD1 based therapy, the presence of high-risk HLA genotype and pre-treatment evidence of islet autoimmunity that could account for the accelerated development of diabetes.

Results

Demographics and clinical characteristics are detailed in Table 1. Biochemistry, HLA genotype and islet antibodies are summarised in Table 2. Individuals were aged 23-82 years and presented within 1-12 weeks (mean 6.4, median 6) of the first dose of anti-PD-1 treatment with diabetic ketoacidosis (DKA) or symptomatic hyperglycaemia requiring hospital admission. Person 1 had an existing history of autoimmunity (rheumatoid arthritis).

Person 9 had a history of type 2 diabetes treated with metformin, a sulphonylurea and empagliflozin. In four people who developed other irAEs, diabetes was the first irAE to manifest. Person 1 presented 6 weeks following anti-PD1 treatment with DKA and concomitant pan-colitis. Person 5 presented 8 weeks after anti-PD1 treatment with DKA and thyroiditis. Six months later he developed widespread rash and inflammatory polymyositis. Person 6 presented with DKA 6 weeks following anti-PD1 treatment, while thyroiditis and rash occurred 6 months later. Person 8 was diagnosed with DKA 12 weeks following treatment and hypophysitis 6 weeks later. Three people received prior anti-CTLA4 therapy. There was no significant difference in diabetes presentation compared to those treated with PD1 inhibitor monotherapy. Person 7 was treated with glucocorticoids in an attempt to salvage β-cell function, with no improvement in glycaemia. A full report of this case has been published by Aleksova and colleagues (19).

Table 1 Demographics and clinical characteristics

Patient	Age/sex	Tumour type	Tumour stage	Immune check point inhibitor	Onset of diabetes since first Anti-PD1 exposure	RECIST Response/duration of response
1	70/M	Melanoma	IV M1b	Pembrolizumab	6 weeks	PR/20 months (Treatment discontinued at 6 weeks)*
2	82/M	SCC (oropharynx)	IV (T2N2C)	Pembrolizumab + carboplatin/5FU	9 weeks	SD/21 months (Treatment ongoing)
3	61/M	Melanoma	Resected IIIC	Pembrolizumab	8 weeks	SD/14 months (Treatment discontinued 8 weeks)*
4	53/F	Melanoma	IV M1b	Pembrolizumab +/- epacadostat	3 weeks	PR/8 months (Treatment ongoing)
5	23/M	Melanoma	IV M1b	Pembrolizumab	8 weeks	PR/21 months (Treatment discontinued at 7 months)*
6	61/M	Melanoma	IV M1b	Sequential ipilimumab, then pembrolizumab	6 weeks	PR/24 months (Treatment ongoing)
7	60/M	Melanoma	IV M1c	Sequential ipilimumab, then pembrolizumab	5 weeks	CR/29 months (Treatment discontinued at 1.5 months)*
8	50/M	Melanoma	IV M1c	Ipilimumab and nivolumab	3 months	SD/5 months (Treatment ongoing)
9	65/M	Melanoma	IV M1c	Pembrolizumab	2 days	Not restaged/1.5 months (Treatment withdrawn)*

CR, complete response; F female; M, male; PD, progressive disease; PD1, programmed cell death protein-1; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumours; SCC, squamous cell carcinoma; SD, stable disease.
*Persons 1, 3, 5, 7 and 9 discontinued treatments at the time of diagnosis of Type 1 diabetes mellitus. They maintained the responses they had achieved despite no additional doses of anti-PD1 therapy. Person 3 received treatment in the adjuvant setting. He discontinued pembrolizumab after 8 weeks and remained disease free for a further 7 months before developing disease progression in the brain. Patient 5 discontinued pembrolizumab at 7 months because of several immune-related toxicities including myositis and Type 1 diabetes. He maintained the partial response for 21 months and has now progressed with intracranial metastases.

Antibodies to glutamic acid decarboxylase (GAD), tyrosine phosphatase-related islet antigen 2 (IA-2), insulin and zinc cotransporter 8 (ZnT8) were measured. GAD antibody was positive

in six out of nine people. Pre-treatment testing in person 4 identified GAD antibodies >1000 U/mL (reference range <5). Conversely, person 8 had undetectable pre-treatment GAD antibodies and demonstrated seroconversion 12 weeks post treatment to 7.6 U/mL. HLA typing was performed in 6 of the 9 people in our series. All had at least one high risk class II HLA allele (DR3, DR4, DQ2 or DQ8). Person 6 had both susceptibility and resistance haplotypes.

Table 2 Diabetes presentation

Patient	Relevant background	Diabetes presentation	Other immune toxicities	Biochemistry‡	HbA1c	Antibodies	HLA§
1	CVID Rheumatoid arthritis	DKA	Colitis	Glucose 50 mmol/l Serum ketones 6 mmol/l pH 6.99	73.8 mmol/mol (8.9%)	GAD >2000 U/mL (<5) IA2 <0.3 U/mL (<15) Insulin Ab 0.3 U (<0.7)	–
2	–	Symptomatic hyperglycaemia with ketosis	Hypothyroidism, rash, arthritis	Glucose 36mmol/l Urinary ketones ++ C peptide <0.03 nmol/l	69.4 mmol/mol (8.5%)	GAD <0.6 U/mL (<5) IA2 <0.3 U/mL (<15) Insulin Ab 0.3 U (<0.7) ZnT8 Ab 0.2 U (<3.1)	DRB1*04 (DR4) DQB1*03:02 (DQ8)
3	–	DKA	–	Glucose 15 mmol/l Serum ketones 5.8 mmol/l pH 7.30 C peptide <0.03 nmol/l	60.7 mmol/mol (7.7%)	GAD >2000 U/mL (<5) IA2 <0.3 U/mL (<15) Insulin Ab 0.3 U (<0.7) ZnT8 Ab 0.3 U (<3.1)	DRB1*04 (DR4)
4	–	DKA	–	Glucose 34 mmol/l Serum ketones 5.8 mmol/l pH 7.09 C peptide 0.04 nmol/l	55.2 mmol/mol (7.2%)	GAD >2000 U/mL (<5) IA2 <0.3 U.mL (<15) Insulin Ab 0.4 U (<0.7)	DRB1*03 (DR3) DRB1*04 (DR4) DQB1*03:02 (DQ8)
5	–	DKA	Thyroiditis (thyrotoxic phase followed by hypothyroidism), rash, inflammatory polymyositis	Glucose 41.4 mmol/l Urinary ketones ++ pH 7.20 C peptide 0.11 nmol/l	58 mmol/mol (7.5%)	GAD 12.0 U/mL (<1.45) IA2 <1.1 U/mL (<1.1) Insulin Ab 1.2 U (0-0.7)	DRB1*03 (DR3) DRB1*04 (DR4) DQB1*03:02 (DQ8)
6	Large pancreatic metastasis	DKA	Immune rash and thyroiditis	Glucose 15.1 mmol/l Serum ketones present pH 7.14 C peptide 0.12 nmol/l	70 mmol/mol (8.6%)	GAD >2000 U/mL (<5) IA2 <1.1 U/mL (<10) ZnT8 <15 (<15)	DRB1*03 (DR3) DRB1*15 (DR15) DQB1*06 (DQ6)
7	Prostate cancer	DKA	–	Glucose 27 mmol/l Blood ketones 5.9 mmol/l C peptide 0.057nmol/l	62 mmol/mol (7.8%)	GAD <0.6 U/mL (<5) IA2 <0.3 U/mL (<15)	–
8	–	DKA	Hypophysitis (adrenal and thyroid axis)	Glucose >41.5 mmol/l Serum ketones 6.1 pH 7.04 C peptide 0.01nmol/l	43 mmol/mol (6.3%)	GAD 7.7 U/mL (<5) IA2 <0.3 U/mL (<15)	DRB1*04 (DR4) DQB1*03:02 (DQ8)
9	Type 2 diabetes on an SGLT2 inhibitor	DKA	–	Glucose 24 mmol/l Serum ketones 6 mmol/l pH 7.24 C Peptide 0.38 nmol/l	57 mmol/mol (7.4%)	GAD <0.6 U/mL (<5) IA2 <0.3 U/mL (<15)	–

CVID, Common variable immune deficiency; DKA, diabetic ketoacidosis; GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; IA2, islet antigen 2; SGLT2, sodium glucose co-transporter- 2; ZnT8, zinc co-transporter 8.
‡Biochemistry reference ranges: Glucose 4–6 mmol/l, serum ketones <0.6 mmol/l, C peptide 0.30-2.40 nmol/l.
§HLA: DR3 and DR4: strongly associated with diabetes, DQ8: linked to juvenile diabetes, coeliac disease and rheumatoid arthritis, DR15 and DQ6: resistance to diabetes

The 28 previously published cases between 2015-2017 are summarised in Table 3.

Table 3 Review of cases published to date

Author/ year published	Age/ gender	Tumour Type	Onset of diabetes	Diabetes presentation	Antibody and HLA status† (As reported)
Hughes *et al*, 2015 [10]	55/F	Melanoma	5 months	DKA	Antibody negative HLA A2.1+, DR4+
	83/F	Non-small-cell lung cancer	< 1 month	DKA	Antibody positive (GAD only) HLA A2.1+, DR4+
	63/M	Renal cell carcinoma	4 months	Elevated random glucose	Antibody positive (GAD, ICA512, Insulin Ab) HLA A2.1+, DR4+
	58/M	Small cell lung cancer	1 week	DKA	Antibody positive (GAD only) HLA A2.1+
	64/F	Melanoma	< 1 month	Elevated glucose with ketonuria	Antibody negative HLA DR4+
Mellati *et al*, 2015 [11]	70/M	Adenocarcinoma (lung)	15 weeks	Hyperglycaemia followed by DKA	Antibody negative HLA not known
	66/F	Sarcomatoid squamous cell carcinoma (jaw)	7 weeks	DKA	Antibody positive (GAD only) HLA DR3-DQ2/DR4-DQ8
Martin-Liberal *et al*, 2015 [12]	54/F	Melanoma	After 3rd infusion	Hyperglycaemia	Antibody positive (GAD only) HLA A2 DR4 DQ8
Gaudy *et al*, 2015 [13]	44/F	Melanoma	2 weeks after second dose	DKA 'Fulminant Type 1 diabetes'	Antibody negative No high risk HLA haplotype
Chae *et al*, 2016 [14]	76/M	Adenocarcinoma (lung)	1 month	Asymptomatic hyperglycaemia	Antibody positive HLA not known
Godwin *et al*, 2016 [15]	34/F	Non-small-cell lung cancer	4 weeks	DKA	Antibody positive (GAD, IA2, Insulin Ab) No high risk HLA
Thoreau *et al*, 2016 [16]	73/M	Melanoma	26 weeks	DKA and acute leg ischaemia	Antibody negative HLA not known
Miyoshi *et al*, 2016 [17]	66/F	Melanoma	4 months	DKA 'Fulminant Type 1 diabetes'	Antibody negative (GAD, IA2, ZnT8) HLA DRB1 HLA DQB1
Lowe *et al*, 2016 [18]	54/M	Melanoma	4 months	DKA 'Fulminant Type 1 diabetes'	Antibody positive (GAD only) HLA-I A2 and HLA-II DQB1*0602
Okamoto *et al*, 2016 [20]	55/F	Melanoma	12 months	Elevated glucose with ketonuria 'Fulminant Type 1 diabetes'	Antibody negative HLA DRB1*04:05-DQB1*04:01
Hansen *et al*, 2016 [21]	58/M	Melanoma	51 weeks	Symptomatic hyperglycaemia	Antibody positive (GAD) HLA not known
Teramoto *et al*, 2016 [22]	63/F	Melanoma	30 weeks	DKA	Antibody negative (GAD, IA2 and Insulin) HLA not known
Fukui *et al*, 2016 [23]	62/F	Melanoma	130 days	Symptomatic hyperglycaemia 'Fulminant Type 1 diabetes'	Antibody negative (GAD and IA2) HLA DRB1*13:02 and DQB1*06:04
Matsumura *et al*, 2016* [24]	68/M	Adenocarcinoma (lung)	40 days	Severe hyperglycaemia with low c peptide	Antibody negative (GAD HLA A*24:02 and DRB1*09:01 and DRB1*15:02
Shah *et al*, 2016 [25]	77/F	Squamous cell carcinoma (lung)	10 days	Hyperglycaemia and ketosis	Antibody negative HLA-A2, HLA-DR4 both negative
Munakata, *et al*, 2016 [26]	72/M	Hodgkin lymphoma	57 Days	Hyperglycaemia 'Fulminant Type 1 diabetes'	Antibody negative HLA-B*40:02
Asai, *et al*, 2017 [27]	50/M	Adenocarcinoma (lung)	After 5th cycle	Hyperglycaemia 'Fulminant Type 1 diabetes'	Antibody negative HLA not known

Table 3 (*Continued*)

Author/ year published	Age/ gender	Tumour Type	Onset of diabetes	Diabetes presentation	Antibody and HLA status† (As reported)
Li *et al* 2017 [28]	63/M	Squamous cell carcinoma (lung)	27 days	DKA	Antibody positive (GAD) HLA not known
Hickmott *et al* 2017 [29]	57/M	Urothelial cancer	After 5th cycle	Hyperglycaemia and ketosis	Not available
Gauci *et al* 2017 [30]	73/M	Melanoma	6 weeks	DKA	Antibody positive (GAD and ZnT8) HLA not known
Usui *et al* 2017 [31]	31/M	Non-small-cell lung cancer	2 weeks	Hyperglycaemia 'Fulminant Type 1 diabetes'	Antibody positive (GAD) HLA DRB1*04:05-DQB1*04:01
	62/F	Non-small-cell lung cancer	10 weeks	Symptomatic hyperglycaemia	Antibody negative HLA DRB1*09:01-DQB1*03:03
Ishikawa *et al* 2017 [32]	54/F	Melanoma	Unavailable	Hyperglycaemia 'Fulminant Type 1 diabetes'	Antibody status and HLA not known

F, female; GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; IA2, islet antigen 2; M, male; ZnT8, zinc co-transporter 8.
*Person with pre-existing Type 2 diabetes.
†HLA A2 DR4 DQ8, HLA DR3-DQ2, HLA A*24:02 and DRB1*09:01, DRB1*04:05-DQB1*04:01: high risk haplotypes for Type 1 diabetes, HLA DRB1*04:05-DQB1*04:01, HLA-B*40:02, HLA DRB1*09:01-DQB1*03:03: associated with Type 1 diabetes in Japan and fulminant diabetes, HLA DRB1*13:02 and DQB1*06:04: weakly associated with Type 1 diabetes, HLA-II DQB1*0602, HLA DRB1*15:02: protective for Type 1 diabetes, HLA DRB1 11:01 13:02:01, HLA DQB1 03:01:01 06:04:01: not associated with Type 1 diabetes.

Discussion

Consistent with other reports, the majority of people in our series presented with DKA within weeks of starting anti-PD1 based therapy. HbA1c tended to be lower at onset in keeping with rapid islet destruction and hyperglycaemia allowing less time for glycosylation of haemoglobin. Individuals exhibited marked glycaemic variability with frequent episodes of hypoglycaemia despite regular insulin adjustment. This rapid onset and fulminant course imply an accelerated islet autoimmunity in contrast to the more protracted kinetics of spontaneous type 1 diabetes in children and young adults. (33,34). It is noteworthy that immunosuppressive doses of corticosteroids are not helpful in reversing islet cell destruction. The result is phenotypic type 1 diabetes, with features of fulminant diabetes described in Japan, Korea and in a case published in Australia (36). Nine of the published cases highlight this distinct phenotype (Table 3). Understanding how PD-1 blockade impacts immune response in individuals with varying autoimmune susceptibility is critically important.

C-peptide was low in most cases despite presenting in less than 12 weeks. Similar rapid progression to diabetes was seen in diabetic patients given islet transplantation from their co-

twin or HLA matched sibling who, given HLA identity, received little or no immunosuppression (37). This observation provided critical evidence that type 1 diabetes is an autoimmune disease. The effect is due to endogenously accumulated islet reactive activated memory T cells in the diabetic sibling which caused rapid graft failure. In PD-1 inhibitor treatment, T cells are activated by exogenous intervention. In both situations, beta cells are exposed to a high number of activated T cells over a short period of time.

The prevalence of GAD antibody positivity in most cases published is in keeping with GAD being the most common antigen specificity in adult-onset type 1 diabetes (38). Person 4 in our series had elevated levels of GAD antibodies pre-treatment and these remained high throughout therapy. This finding was also reported by Gauci (30). In contrast, the GAD antibody titre in person 8 was <0.6 U/ml pre-treatment but elevated at 7.6 U/mL at diabetes presentation. Lowe and colleagues described a similar outcome of GAD antibody seroconversion and development of DKA 2 weeks following the third infusion of combination ipilimumab/nivolumab (18).

Collectively, in our series and other published cases, there were 18 people with elevated GAD antibodies at diabetes presentation and 17 without.

Person 9 had pre-existing type 2 diabetes which was well controlled on triple oral anti-hyperglycaemic agents. Matsumura et al. also described a person with pre-existing diet-controlled diabetes, who developed severe hyperglycaemia with very low C-peptide and persistently negative GAD antibody on Nivolumab (24). Person 9 had been on a stable dose of a sodium-glucose co-transporter-2 (SGLT-2) inhibitor for over 6 months and was well on the day of anti- PD-1 treatment. This individual presented with DKA two days after

Pembrolizumab therapy but remained antibody negative. C peptide was higher at diabetes presentation than in other cases. SGLT-2 inhibitors have been associated with euglycaemic DKA when used to treat both type 1 and type 2 diabetes. The exact contribution of the SGLT-2 inhibitor to the rapid manifestation of DKA remains uncertain. It is plausible that ketosis was accelerated during early autoimmune pancreatic destruction resulting in a relatively preserved c-peptide concentration in this subject. There have been no published cases of DKA in the setting of combined SGLT-2 inhibitor and PD1 inhibitor use. However, caution should be exercised in this cohort particularly as development of DKA two days following immune checkpoint inhibitor administration is the earliest pancreatic irAE reported to date. Unfortunately, HLA typing was not possible in this case but may have been useful in delineating aetiology.

Notably, the six that were HLA typed in our series demonstrated high risk alleles. Preclinical studies support the premise that PD-1 loss may induce specific autoimmune disease depending on pre-existing genetic background (39). In published cases, 14 people had at least one high risk HLA allele, 2 had high risk and protective alleles, 3 had no high-risk alleles and 11 reports did not record HLA (Table 3). The clinical utility of HLA typing and baseline islet antibody testing to stratify risk and manage potential irAEs remains to be prospectively evaluated.

Understanding the underlying mechanisms of irAEs may provide insights and facilitate the development of targeted approaches to deal with the autoimmune side effects of immune checkpoint inhibitors. Recent studies have shown that blocking IL-7 receptor-α (IL-7Rα) with monoclonal antibodies in non-obese diabetic mice has been shown to prevent and reverse autoimmune diabetes by inducing exhaustion of auto-reactive T cells (40). In

humans, Abatacept (CTLA-4 Ig) was found to increase endogenous C-peptide production in newly diagnosed type 1 diabetes and is now being trialled in antibody positive relatives with normal glucose tolerance (41).

In conclusion, PD1 inhibitor-induced diabetes is a rare and irreversible complication. As checkpoint inhibitors become standard care in many tumour types and with the introduction of combination approaches, we anticipate an increased incidence of iatrogenic type 1 diabetes. The clinical phenotype is a steroid unresponsive, accelerated and fulminant islet autoimmunity that usually presents with DKA. People receiving anti-PD-1 therapy should receive education on recognising symptoms of hyperglycaemia to aid early presentation. This series has prompted a prospective study to investigate predictive risk factors including pre-treatment islet antibodies and HLA status. This will clarify whether checkpoint inhibitors are an accelerator, or initiator of type 1 diabetes and guide future management and potentially prevention of this complication. Endocrinologists and oncologists need to be increasingly vigilant with early recognition and prompt treatment for this life-threatening form of diabetes.

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2.3 The local incidence of diabetes and pancreatitis after combination immune checkpoint inhibition: Unpublished data

Introduction

In publication 1, I defined the phenotype of CPI related diabetes as an accelerated, fulminant islet autoimmunity leading to rapid onset insulin deficiency and risk of ketosis. This study reviewed 9 patients presenting with this phenomenon across several tumour streams. Due to the nature of this case series, there was no known denominator from which to draw conclusions about the true local incidence. In line with emerging data from international studies outlined in Chapter 1, I expected the overall incidence to be approximately 1%. With an increasing number of severe toxicities reported in patients treated with combination immune checkpoint inhibition (cICI), little is known about the risk of diabetes with the addition of anti-CTLA-4 to anti-PD-1[29].

Aims

1. To describe the incidence of new onset diabetes or pancreatitis after cICI in patients with advanced melanoma;
2. To describe the pancreatic enzymes and FDG-PET/CT findings in patients diagnosed with diabetes or pancreatitis.

Methods

All patients treated with cICI for melanoma between January 2016- January 2019 were identified via pharmacy records. Medical records were reviewed to identify patients with documented immune related adverse events. In regard to diabetes and pancreatitis, plasma glucose concentration, HbA1c, lipase, and islet autoantibodies (where measured by the treating clinician) were collected. Positron Emission Tomography with 2-deoxy-2-[^{18}F]

fluoro-D-glucose combined with computed tomography (FDG-PET/CT) images were retrospectively reviewed by a nuclear medicine physician with specific reference to the pancreas in all patients with available baseline and post-treatment imaging. A diffuse increase in the pancreatic standardised uptake value (SUVmax) was considered suspicious for a pancreatic irAE. A detailed description of the methods is outlined in Chapter 1.8.

Results

Patient characteristics

Overall, 162 patients were treated with cICI in the study period. The median age was 60 years (IQR 49-69) and 30% of the cohort were female. 50 (31%) had received a prior line of CTLA-4 and/or PD-1 monotherapy and 56 (35%) had received targeted immunotherapy with combination BRAF/MEK inhibition (Table 1.)

Table 1. Baseline Characteristics

Characteristic	**No. (%)** **Whole cohort n=162**
sex	
Male	113 (70%)
Female	49 (30%)
Age (years)	60 (48.7-69.3)
BMI (kg/m^2)	27 (24.2-30.2)
ECOG status at baseline*	
0	94 (58%)
1	24 (15%)
2	4 (2%)
3	1 (0.6%)
missing	42 (26%)
Smoking status	
Never smoked	45 (28%)
Ex-smoker	23 (14%)
Current smoker	11 (7%)
Missing	83 (51%)
Prior exposure to any ICI	50 (31%)
Anti-CTLA-4 monotherapy	9 (<1%)
Anti-PD-1/PD-L1 monotherapy	46 (28%)
Prior exposure to other treatment	49 (30%)
Chemotherapy	14 (7%)

BRAF/MEK inhibition	56 (35%)
Missing	51 (32%)
Prior autoimmunity	
Total	17 (11%)
Rheumatoid arthritis	4 (2%)
Inflammatory bowel disease	4 (2%)
Psoriasis	2 (1%)
Vitiligo	1 (0.6%)
Alopecia	1 (0.6%)
Sjögrens' syndrome	1 (0.6%)
Graves' disease	1 (0.6%)
Hashimoto's thyroiditis	1 (0.6%)
Coeliac disease	1 (0.6%)
Type 1 diabetes	1 (0.6%)
Multiple sclerosis	1 (0.6%)
Behcet's disease	1 (0.6%)

*Eastern Cooperative Oncology Group (ECOG) is a performance status scoring system from 0 (perfect health) to 5 (death).

Immune related adverse events

135 (83%) developed at least one irAE, which was severe or life-threatening (Grade 3 or 4) in 78 (48%). Individual organ system toxicity is outlined in Table 2. Dermatological toxicity (a composite of rash, vitiligo and alopecia) was the most common form of irAE with an incidence of 41%, followed by gastrointestinal (gastritis/enteritis/colitis) and hepatitis with an incidence of 29% and 28% respectively. Of the 162 patients treated with cICI, there were no definite cases of autoimmune diabetes detected. In general, screening glucose readings and HbA1c were not performed without clinical suspicion. Lipase levels were added to liver function tests on an ad hoc basis, determined by the treating clinician.

Two individuals were noted to have new onset hyperglycaemia requiring insulin which was attributed to high dose glucocorticoids prescribed for another irAE. One had an undetectable C peptide raising suspicion for CPI-DM, and a non-elevated GAD antibody. The other was not evaluated for islet autoimmunity. A third patient had an incidental finding of a diabetic range HbA1c 7.5% (58mmol/mol) on screening bloods, which was not investigated further

by the treating team. A self-limited asymptomatic rise in lipase was detected in six individuals.

Table 2. Immune Related Adverse Events (irAEs)

Event	**No. (%)**
One or more irAE	135 (83%)
Grade 3 / 4 irAE	78 (48%)
Glucocorticoid treatment required for an irAE	100 (62%)
Unplanned emergency or hospital presentation for an irAE	84 (52%)
Endocrine irAEs	
Type 1 diabetes	0 (0%)
Asymptomatic elevated lipase	6 (3.7%)
Hypophysitis	31 (19%)
Thyroiditis	43 (27%)
Non-endocrine irAEs	
Skin/hair	66 (41%)
Gastrointestinal	47 (29%)
Hepatitis	46 (28%)
Rheumatic	19 (12%)
Pneumonitis	15 (9%)
Neurological	6 (4%)
Haematological	6 (4%)
Nephritis	4 (2%)
Myocarditis	1 (<1%)

FDG-PET/CT Findings

FDG-PET/CT at baseline and within the first 6 months of cICI treatment was available in 134/162 patients. A diffuse increase in pancreatic SUVmax was found in six patients, including 2/3 patients with new onset hyperglycaemia, 3/6 with an asymptomatic rise in lipase, and in an additional one patient who was diagnosed with a myriad of irAEs requiring prolonged high dose glucocorticoids, but who did not have documented hyperglycaemia or an elevated lipase. In 2/6 patients with increase pancreatic uptake on FDG-PET/CT, interval pancreatic atrophy was later observed on follow up CT. Diabetes investigations, lipase and FDG-PET/CT findings are summarised in Table 3.

Table 3. A summary of biochemical and FDG-PET/CT features in patients with suspected diabetes or pancreatitis

Patient	Diagnosis	Diabetes Investigations	Pancreas SUV (18F-FDG PET)	Pancreas size (CT)
1	New hyperglycaemia after glucocorticoids	GAD Ab negative HbA1c 11.7% C peptide low	Normal	No significant change
2	New hyperglycaemia after glucocorticoids	HbA1c, C peptide, antibodies and lipase not measured	SUVmax 6.4 Diffuse increase in uptake	Mild increase in the size at the time of pancreatitis
3	Asymptomatic elevated lipase	HbA1c of 7.5% C peptide and antibodies not measured	SUVmax 5.4 Diffuse increased uptake	At the time of pancreatitis the pancreas became larger and amorphous Later the pancreas became small and atrophic
4	Asymptomatic elevated lipase	One normal BSL HbA1c and antibodies not measured	SUVmax 6.0 Diffuse increased uptake	No significant change
5	Asymptomatic elevated lipase	None	SUVmax 3.2 Mild diffuse uptake	No significant change
6	Asymptomatic elevated lipase	None	SUVmax 3.1 Mild diffuse uptake	No significant change
7	Asymptomatic elevated lipase	None	PET not available	No significant change
8	Asymptomatic elevated lipase	None	Normal	No significant change
9	Myriad of irAEs requiring high dose glucocorticoids No elevated lipase	None	SUVmax 6.1 Diffuse increased uptake	Significant reduction in the size of pancreas later seen

Legend: Abnormalities relating to glycaemia or pancreatic imaging are highlighted in red.
Abbreviations: Immune related adverse events (irAEs), Glutamic Acid Decarboxylase Antibody (GAD Ab), Haemoglobin A1c (HbA1c), Blood sugar level (BSL), maximum Standardised Uptake Value (SUVMax), ^{18}F-labeled fluoro-2- deoxyglucose Positron emission tomography (18F-FDG-PET)

Discussion

In this cohort of 162 patients treated with cICI for advanced melanoma, there were no cases of definite checkpoint inhibitor related autoimmune diabetes mellitus (CPI-DM). Of interest were the six patients with diffusely increased FDG uptake in the pancreas after treatment commenced, two of whom did have evidence of new hyperglycaemia and four had evidence of subacute exocrine pancreatic inflammation in the form of a raised lipase. Whether the increased FDG uptake reflects exocrine tissue inflammation or generalised pancreatic inflammation is not known. No patients developed symptomatic pancreatitis (abdominal pain, acute illness) or fulminant exocrine pancreatic failure (malabsorption, steatorrhea). Varying degrees of endocrine and exocrine dysfunction with or without features of islet autoimmunity and with associated changes on pancreatic imaging have been described in more recent literature.

Marchand and colleagues reported a case involving a 55-year-old male treated with nivolumab for pulmonary pleomorphic carcinoma. After 9 cycles, the patient presented with diabetic ketoacidosis (DKA) with a HbA1c at diabetes onset of 8.2% (66.1mmol/mol). Islet autoantibodies to islet cell (ICA), glutamic acid decarboxlyase (GAD), insulinoma-associated protein 2 (IA-2), islet-specific zinc transporter 8 (ZnT8), and insulin were all negative. The class II HLA haplotype (DR-12-DQ7/DR15-DQ6) was not associated with increased risk of type 1 diabetes. In response to a mixed meal test, the C peptide was undetectable and glucagon response was blunted, suggestive of alpha and beta cell dysfunction. While lipase was not reported and no gastrointestinal symptoms emerged, a random faecal elastase was low consistent with subclinical exocrine dysfunction. Abdominal CT imaging during the course of treatment was then retrospectively evaluated. After 4 cycles of treatment, before onset of DKA there was an initial 15% increase in pancreatic volume suggestive of an inflammatory response. Gradual interval reduction in pancreatic volume was then demonstrated on serial imaging, with a 63% reduction from baseline pancreatic volume evident 3 months after the presentation with DKA[76]. To summarise, this case reflects antibody negative fulminant CPI-DM, subclinical exocrine pancreatic dysfunction and significant pancreatic atrophy after treatment with a PD-1 inhibitor.

Dehghani and colleagues described a more atypical series of events. A 63-year-old male was treated with nivolumab for metastatic melanoma. After 15 months of treatment, a surveillance FDG-PET/CT scan incidentally detected an intense focal area of increased uptake within the tail of the pancreas. A dedicated pancreatic MRI was performed. The combination of both imaging modalities was suggestive of focal type II autoimmune pancreatitis (non-IgG4 associated fibro-inflammatory pancreatitis). At the time of the

abnormal imaging, serum lipase, IgG4 and glucose concentrations were within normal range. 3 months later, a screening fasting glucose became elevated at 11mmol/L with a significantly elevated HbA1c of 9.3% (78mmol/mol). Despite the diabetic range fasting glucose and HbA1c, a fasting c peptide remained detectable at 0.41nmol/L (reference range 0.27-1.27) and islet autoantibodies to GAD, IA2, ZnT8 and insulin were undetectable. Basal insulin and metformin were commenced. Further investigation revealed a serum lipase elevated to three times the upper limit of normal, with a low faecal elastase suggestive of subclinical exocrine failure. Over time, follow up MRI imaging demonstrated a 50% loss of pancreatic volume and the patient later developed overt exocrine failure with weight loss, profuse diarrhoea and an undetectable faecal elastase. A full antibody panel was repeated including islet, coeliac disease and autoimmune liver disease associated antibodies which were negative. On a repeat mixed meal test, the glucose incremented from 5.9 to 11.2 mmol/L with a corresponding C peptide rise of 0.1 to 0.5nmol/L indicating impaired but not abolished endogenous insulin production. The patient ultimately required a basal bolus insulin regimen and pancreatic enzyme replacement[77]. In summary, this case demonstrates the unusual sequence of imaging changes of focal pancreatitis with initially normal endocrine and exocrine gland function, followed by gradual onset antibody negative islet failure, delayed overt exocrine failure and marked pancreatic atrophy.

Pancreatic atrophy in all patients with spontaneous type 1 diabetes is well described, however the mechanism is not understood. Loss of pancreatic volume may relate in part to the destructive insulitis and reduction of B cell mass with resultant loss of trophic effect of insulin on acinar cells[78]. The additional element of diffuse pancreatic inflammation and exocrine failure after ICI may exacerbate this phenomenon[77].

Conclusion

While none of the 6 patients with pancreatic imaging changes in our cohort developed acute DKA or overt exocrine failure, two had evidence of new hyperglycaemia and 3 had subclinical exocrine dysfunction. A lack of longitudinal testing of autoantibodies, endocrine and exocrine function in these patients resulted in insufficient evidence to diagnose CPI-DM in this cohort. Although not universally observed, temporal changes in pancreatic volume and FDG metabolism provides further evidence for an underlying irAE pancreatitis in patients who may present atypically compared to the previously defined CPI-DM. These findings prompt the recommendation that longitudinal assessment of pancreatic endocrine and exocrine function including fasting glucose, c-peptide, lipase and faecal elastase should be undertaken in patients with an incidental finding of pancreatic inflammation or atrophy on imaging. In the event of new onset diabetes, islet autoantibodies should be assessed although their absence does not exclude the possibility of CPI-DM.

2.4 Classic and non-classic presentations of diabetes after immune checkpoint inhibition: A case-based discussion

Introduction

Chapters 2.2 and 2.3 have highlighted differential phenotypes of pancreatic immune related adverse events. The importance of recognising the underlying cause for new onset dysglycaemia in order to provide appropriate education and management to the patient is becoming increasingly evident.

The delineation between checkpoint inhibitor associated autoimmune diabetes mellitus (CPI-DM) and other causes of hyperglycaemia becomes ever more relevant when considering therapeutic options. At present, there are no prospective studies investigating a drug for reversal of β-cell destruction following ICI. Glucocorticoids have not been effective and permanent insulin dependent diabetes follows[37]. Recently, a report was published describing reversal of CPI-DM with the TNF-alpha inhibitor, infliximab[75]. In this case, confounding factors such as recent intra-articular steroid use and evidence of impaired insulin sensitivity raise doubt over the mechanism of response to infliximab. Whilst it is timely to consider immune therapies for this iatrogenic form of diabetes, we must first be sure the patient has autoimmune diabetes.

In 2019, I was a finalist in the Australian Diabetes Society Clinical Science Poster Award for a presentation titled “Classic and non-classic presentations of immunotherapy related type 1 diabetes” (Appendix 1.). This work briefly summarised the features of acute CPI-DM followed by 3 atypical case examples from patients treated at our centres. I was then involved in a manuscript titled “High dose glucocorticoids masking immune checkpoint inhibitor

diabetes mellitus in two patients treated for metastatic melanoma" (Appendix 2.). This report raised the need for increased vigilance for the possibility of CPI-DM in glucocorticoid treated patients to avoid development of DKA and associated morbidity. This chapter summarises 4 case-based presentations from Appendices 1 and 2, aiming to highlight specific features within atypical cases which may or may not support an underlying diagnosis of CPI-DM.

Case 1.

A 65-year-old female was treated with combination PD-1 inhibition (tislelizumab) and BTK inhibitor (zanubrutinib) for relapsed lymphoma. A prior history of coeliac disease, psoriasis, elevated lupus anticoagulant antibodies and hypogammaglobulinemia was noted. 30 months after initiation of treatment, an FDG-PET/CT demonstrated the incidental finding of diffuse uptake throughout the pancreas (Appendix 1). Diabetic range glycaemia was noted at the time (fasting glucose 11.8 mmol/L, HbA1c 7.6% (59nmol/L)), however the C peptide was not suppressed at 0.79 nmol/L (reference range 0.4-1.5), islet autoantibodies were undetectable, and the lipase was normal. Target glycaemia was achieved with oral metformin and a sulphonylurea. Four months after the initial presentation, islet antibodies were measured again which demonstrated late GAD antibody seroconversion to 42 IU/ml (reference range <10). The C peptide remained non-supressed at 0.61nmol/L and glucose at target. On repeat FDG-PET/CT, the pancreas had become small and amorphous. The subacute presentation and late GAD seroconversion in this case may be similar to that of latent autoimmune diabetes in adults (LADA), and the strong personal history of autoimmunity was also of relevance. The patient was provided education regarding glucose monitoring and risk of ketosis and was referred to a specialist diabetes clinic to monitor need for introduction of insulin.

Case 2.

A 65-year-old male was treated with the PD-1 inhibitor pembrolizumab for metastatic melanoma. He had a prior diagnosis of type 2 diabetes managed with metformin, a sulphonylurea and a sodium-glucose co-transporter 2 (SGLT-2) inhibitor. Two days after the first dose of pembrolizumab, he was admitted with diabetic ketoacidosis. At presentation, the C peptide was not suppressed at 0.38nmol/L (reference range 0.4-1.5) and islet antibodies were negative. The patient became increasingly unwell and was transferred to the palliative care unit. Whether this presentation was due to CPI-DM or the SGLT-2 inhibitor, known to cause ketoacidosis, is unknown. Patients taking SGLT-2 inhibitors are now routinely advised to cease the medication while unwell, fasting or before surgery and monitor ketones to avoid this complication[79].

Case 3.

A 65-year-old man was treated with cICI (ipilimumab and pembrolizumab) for metastatic melanoma. 15 months after initiation of treatment, he required a two-month course of high-dose glucocorticoids for Grade III pneumonitis. Two months after glucocorticoid cessation, he was admitted with acute respiratory symptoms. A random blood glucose concentration was elevated at 21.5mmol/L with a detectable C peptide of 0.3nmol/L (reference range 0.4-1.5). The serum ketones were elevated at 5.2mmol/L (reference range <0.3). The acid base analysis was suggestive of a respiratory acidosis with a normal bicarbonate, atypical for DKA as the driving mechanism (Appendix 1). Islet antibodies were negative, and no high-risk class II HLA allele was expressed. The contribution of recent high dose glucocorticoids and acute illness in the onset of hyperglycaemia and ketosis in this patient is not known but CPI-DM was not excluded. This patient was provided education about CPI-DM and risk of ketoacidosis and was referred to a specialist diabetes clinic for close monitoring.

Case 4.

A 71-year-old male was treated with cICI (ipilimumab and nivolumab) for metastatic melanoma. The first treatment cycle was complicated by enterocolitis requiring a short course of pulsed intravenous methylprednisolone and commencement of the TNF-alpha inhibitor infliximab. At the time, an FDG-PET scan demonstrated an incidental finding of diffusely increased uptake in the pancreas (Appendix 2.). The pancreatic enzymes were transiently elevated and random BSLs measured between 7-10.9mmol/L without glucose lowering therapy. 6 months later, progressive intracranial disease necessitated re-introduction of long-term high dose glucocorticoids. Soon after, the patient presented with severe DKA requiring an intensive care admission. The C peptide was mildly suppressed but islet autoantibodies were negative. Follow-up FDG-PET/CT images demonstrated significant interval pancreatic atrophy over time. Although the episode of severe hyperglycaemia was no doubt precipitated in part by the high dose glucocorticoid, the presence of underling pancreatic inflammation on FDG-PET/CT and the severe DKA presentation suggests an underlying diagnosis of subacute CPI-DM.

Conclusion

The defining feature of CPI-DM is autoimmune destruction of the pancreatic islets ultimately leading to insulin deficiency. In patients who lack the acute fulminant presentation described in Publication 1, clues to the presence of CPI-DM may include a history of autoimmunity, delayed islet antibody seroconversion or evidence of pancreatic inflammation on FDG-PET/CT. Patients receiving treatment with a PD-1/PD-L1 inhibitor may benefit from routine monitoring of plasma glucose concentration and lipase so as to detect and monitor changes early. As highlighted in Chapter 2.3, new onset hyperglycaemia in these patients should

prompt longitudinal surveillance of C peptide and islet autoantibodies to identify those at risk of diabetic ketoacidosis and insulin dependence.

2.5 Publication 2: Comment Letter

Comment on Trinh et al. Successful Treatment of Immune Checkpoint Inhibitor–Induced Diabetes Mellitus with Infliximab.

Diabetes Care 2019;42:e1 DOI: 10.2337/dc19-1747

Comment on Trinh et al. Successful Treatment of Immune Checkpoint Inhibitor–Induced Diabetes Mellitus with Infliximab. Diabetes Care 2019 Jul; dc190908.

Short running title: Trinh et al. comment letter

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Trinh et. Al (1) have described normalisation of plasma glucose concentrations coinciding with administration of the tumour necrosis factor (TNF)-alpha inhibitor infliximab in a patient who had developed diabetes after immune checkpoint inhibitors (CPI).

Diabetes associated with PD1/PDL1 inhibition is a significant complication occurring in around 0.9% of patients. The classic presentation is acute rise in blood glucose concentration with relatively low HbA1c and steroid-unresponsive progression to permanent islet failure. In a cohort of 64 patients, C peptide was suppressed in more than 90% and GAD autoantibody was present in approximately half. 62% expressed the high-risk class II HLA allele, HLA-DR4 (2).

The case reported by Trinh et al is atypical. Presentation was late and occurred after intra-articular steroid injections (known to cause systemic side-effects such as diabetes). An anti-islet cell IgG titre was 1:100 at baseline, presumably measured by the now superseded indirect immunofluorescence assay. HLA and autoantibodies to GAD, IA-2 and zinc transporter-8 were not reported meaning there was no other evidence for islet autoimmunity. Baseline insulin and c-peptide responses to a mixed meal tolerance test were increased suggestive of peripheral insulin resistance, as might be observed with steroid-induced diabetes. It is unclear if the first test was carried out on insulin treatment. Infliximab then coincided with a decrease rather than increase in plasma c-peptide level. These data, as the authors themselves suggest, indicate reversal of insulin resistance rather than reversal of insulin deficiency.

Infliximab and other biologics are routinely used for the treatment of immune related adverse events such as colitis and arthritis.

In adults with pre-existing GAD antibodies, anti-TNF preceded new onset type 1 diabetes (T1D) and increased antibody titre suggesting that the drug was unable to prevent progression and may have played a role in promoting autoimmunity (3). In a pilot study of 18 children with newly diagnosed T1D, etanercept resulted in lower HbA1c and insulin requirements (4). A randomized controlled trial in stage 2 ("pre-symptomatic") T1D using the fully human anti-TNF monoclonal antibody golimumab is in progress (5). Reports of improved insulin sensitivity and hypoglycaemia in patients with T1D and type 2 diabetes after anti-TNF presumably reflect upregulation of glucose transporter mechanisms (6). This mechanism may explain the normalization of plasma glucose in the reported patient.

Prior to this report, there have been no published data regarding the role of immune modulation for CPI related diabetes and the most appropriate agent is not known. Similarly, no drugs are licensed for the prevention or reversal of spontaneous T1D.

It is timely to consider the role of immune therapies for CPI related diabetes and TNF inhibition may turn out to be useful; however, it is not sufficiently clear that the case reported by Trinh et al demonstrates reversal of autoimmune diabetes. Therefore, it is not a basis for considering the clinical use of anti-TNF for more typical cases.

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2.6 Conclusion

PD-1 inhibitors can induce a rapid autoimmune islet failure suggesting a narrow window for therapeutic intervention. The occurrence of islet antibody seroconversion can be seen in approximately 50% of cases and is associated with an increased risk of DKA[29]. In addition, others have described elevation in GAD autoantibodies before treatment as well as post treatment seroconversion[37], indicating that ICI may both induce de novo autoimmunity and reinvigorate an existing dormant or exhausted immune response. HLA DR4 is present in the majority of patients. In light of emerging evidence of atypical presentations, I suggest that three diabetes phenotypes may occur after ICI;

1. Acute autoimmune CPI-DM: Early onset, acute hyperglycaemia with insulin deficiency manifest by a low C peptide and ketosis risk, associated with the presence of islet autoantibodies or the expression of high-risk class II HLA alleles;
2. Subacute CPI-DM: Insidious onset hyperglycaemia which may be associated with pancreatic inflammation on FDG-PET/CT, other features may include pancreatic atrophy and late islet antibody seroconversion;
3. Non-autoimmune diabetes: Hyperglycaemia that may be associated with other factors such as glucocorticoid induced diabetes, pancreatic metastases or exocrine pancreatitis. Patients with existing type 2 diabetes treated with SGLT-2 inhibitors are at increased risk of ketoacidosis through drug-related mechanisms.

Reversal of CPI-DM would be of great benefit in this otherwise chronic and often brittle form of insulin dependent diabetes. Prospective studies reviewing the role of immunomodulation could be considered in patients with evidence of acute or subacute islet autoimmunity. In patients with other factors such as glucocorticoid treatment, the risks of immunomodulation would need to be weighed against the perceived benefits. In the interim, all patients with

suspected CPI-DM require diabetes education surrounding management of hypoglycaemia, sick day action plans and complication screening.

Chapter 3. Immune checkpoint inhibitor related hypophysitis

3.1 Introduction

The past 8 years have seen a singularly dramatic increase in hypophysitis presentations after treatment with immune checkpoint inhibitors (ICI), particularly in association with CTLA-4 blockade. Unlike primary adrenalitis which can present with an Addisonian-like crisis, the clinical syndrome of hypophysitis after ICI appears to be more insidious with non-specific symptoms such as fatigue, malaise and headache often preceding the diagnosis. In large phase III clinical trials investigating safety and efficacy of cICI compared to single agent ipilimumab and nivolumab, routine monitoring of cortisol and other pituitary hormones was generally not mandated in study protocols[9]. In this chapter, clinical, biochemical and radiological features of hypophysitis in patients treated with cICI are described.

First, I present the results of a retrospective evaluation of the clinical and radiological diagnosis of hypophysitis in a manuscript submitted for publication. In an additional segment, I describe survival data omitted from the manuscript due to word limit restrictions. I conclude the chapter with recommendations regarding differentiating ICI related hypophysitis from other pituitary pathologies seen in cancer patients, as described in a pictorial essay prepared for Clinical Radiology (Appendix 3).

3.2 The complementary role of routine cancer imaging in identifying clinically-occult hypophysitis after combination immune checkpoint inhibition

Manuscript prepared for journal submission

TITLE PAGE

The complementary role of routine cancer imaging in identifying clinically-occult hypophysitis after combination immune checkpoint inhibition

Running title: Increased detection of ICI induced hypophysitis by imaging

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Declarations

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Authors' contributions: SS oversaw the study as Principal Investigator. AG, AI and AL were responsible for study concept and design, drafted the manuscript and take responsibility for the integrity of the data. AG and RW collected the clinical and biochemical data. AI, AL and RH were responsible for acquisition and interpretation of imaging data. AG and AI performed the statistical analyses. All authors interpreted the data and provided critical revision of the manuscript.

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Abbreviations

cICI: Combination immune checkpoint inhibition

anti-CTLA-4: Monoclonal antibody targeting cytotoxic T-lymphocyte associated protein 4

anti-PD-1: Monoclonal antibody targeting programmed cell death protein 1

anti-PD-L1: Monoclonal antibody targeting programmed cell death ligand 1

MRI: Magnetic resonance imaging

CT: Computed tomography

FDG-PET/CT: 2-deoxy-2-[^{18}F] fluoro-D-glucose Positron Emission Tomography with computed tomography

irAE: Immune related adverse event

ESMO: European society of Medical Oncology

NMP: Nuclear medicine physician

SUVMax: Maximum standardised uptake value

ACTH: Adrenocorticotropic hormone

LH: Luteinizing hormone

FSH: Follicle-stimulating hormone

TSH: Thyroid stimulating hormone

ABSTRACT

Background: Hypophysitis is reported in 8.5-10.5% of patients receiving combination immune checkpoint inhibition (cICI) but can be a diagnostic challenge. The aim of this study is to assess the complementary role of routine diagnostic imaging performed during the course of therapeutic monitoring of cICI with anti-CTLA-4/anti-PD-1 agents in the identification of hypophysitis and the relationship of imaging findings to clinical diagnostic criteria.

Methods: This retrospective cohort study identified patients treated with cICI between January 2016- January 2019 at a quaternary melanoma service. Medical records were reviewed to identify patients with a documented diagnosis of hypophysitis based on clinical criteria. Available baseline and post treatment structural brain imaging with Magnetic Resonance Imaging (MRI) or computed tomography (CT) of the brain and 2-deoxy-2-[^{18}F] fluoro-D-glucose Positron Emission Tomography with computed tomography (FDG-PET/CT) were assessed retrospectively for a relative change in pituitary size or visually perceptible increase in pituitary FDG uptake temporally attributed to cICI.

Results: 162 patients (median age 60 years, 30% female) were included. Of these, 100 and 134 had serial CT/MRI of the brain and FDG-PET/CT, respectively. 31 patients had a documented diagnosis of hypophysitis and an additional 21 had isolated imaging findings, in total comprising 32% of the cohort. Concurrent use of high-dose glucocorticoids for other irAEs prevented assessment of the pituitary-adrenal axis in 90% of the patients with isolated imaging findings. There were no demographic features associated with hypophysitis. Primary thyroiditis after cICI was significantly more common in patients who developed hypophysitis (36% vs. 28%, $p<0.001$).

Conclusions: Routine imaging performed during therapeutic response assessment may lead to increased identification and more prompt management of cICI-induced hypophysitis. Careful review of the pituitary imaging during follow-up is recommended.
Keywords: Hypophysitis; pituitary gland; immune related adverse events, combination immune checkpoint inhibition; cancer imaging; immunotherapy

BACKGROUND

The incidence of spontaneous hypophysitis is estimated at 1 in 7-9 million people per year[50]. The widespread use of immune checkpoint inhibitors (ICI) against cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) and programmed cell death 1 (anti-PD-1) or its ligand (anti-PD-L1) for a range of advanced cancers in recent years has resulted in a dramatic increase in incidence, particularly with anti-CTLA-4[50, 52-54]. In the landmark CheckMate 067 trial evaluating combination ICI (cICI), the incidence of hypophysitis reported with ipilimumab and nivolumab combination, nivolumab and ipilimumab was 7%, 1% and 4% respectively, suggesting that anti-CTLA-4 therapy is the major driver of this immune-related adverse event (irAE)[9, 55].

The European Society of Medical Oncology (ESMO) guidelines suggest regular monitoring of thyroid function in patients treated with ICI. However, evaluation of the pituitary axes is generally reserved for clinical suspicion of dysfunction[30]. This recommendation may lead to clinically occult cases being missed, as the symptomology may be non-specific or masked by glucocorticoid therapy for concurrent irAEs.[52, 80]. As such, the true incidence of hypophysitis is likely to be underestimated.

At our center, Positron Emission Tomography with 2-deoxy-2-[^{18}F] fluoro-D-glucose combined with computed tomography (FDG-PET/CT) and neuroimaging with either Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) are routinely used for the surveillance of high-risk melanoma patients[81] and for cICI response assessment[82]. The high glycolytic activity of activated-T-cells enables FDG-PET/CT to coincidentally detect an immunotherapy-related inflammatory response in various tissues, including the pituitary[83]. Apart from small case series[82-84], the utility of FDG-PET/CT in detection of

hypophysitis has not been systematically assessed. The complementary role of routine oncologic imaging for detecting irAEs, such as hypophysitis, in addition to monitoring the status of the patient's metastatic disease, remains under-appreciated.

This study aimed to assess the utility of routine diagnostic imaging in identifying cICI-induced hypophysitis with reference to clinical diagnoses based on symptoms and laboratory criteria.

METHODS

Study design and participants

This single center retrospective cohort study was approved by the institutional ethics committee (approval number 17/231R). Pharmacy records were used to identify all patients who received at least one cycle of cICI for metastatic melanoma between January 2016 and January 2019 with a minimum of 6 months follow-up. Routine safety bloods including full blood count, liver function tests, electrolytes and thyroid function were performed every 4-6 weeks. Random cortisol levels were performed at a variable frequency depending on the treating clinician. FDG-PET/CT and neuroimaging with CT or MRI were undertaken as part of routine treatment response assessment, typically every 12-weeks during treatment unless expedited for clinical reasons (Supplemental Information Items 1.1 and 1.2).

Variables and Data Measurement

Patient demographics, adverse events and outcomes were collected by retrospective chart review. In patients with a clinical diagnosis of hypophysitis, symptomatology and available pituitary hormones were recorded. For the purpose of describing biochemical pituitary dysfunction, cortisol deficiency was defined as a morning cortisol <250nmol/L or random

cortisol <150nmol/L and interpretation of other parameters was according to ESMO guidelines[30]. In keeping with clinical guidelines, thyroid function tests (TSH, T3 and T4) were performed at each cycle during cICI and every 4-8 weeks during maintenance nivolumab.

In parallel, all available CT and MRI brain scans were assessed to determine whether the pituitary was adequately imaged. Evaluable MRI brain examinations included fine-slice pre- and post-contrast T1-weighted imaging in the axial plane, with multiplanar reconstructions. Sagittal reconstructions of the post-contrast T1-weighted sequence were used to measure gland size in the plane of the pituitary stalk. In the case of alternative imaging study (CT, or targeted pituitary MRI), the gland size was assessed in the same plane. Pituitary size and appearance before and after treatment were retrospectively assessed by a neuroradiologist who was blinded to clinical information. Imaging criteria for cICI-induced hypophysitis have not been established. For this study, we defined the MRI imaging criteria for imaging detected hypophysitis as 1) a relative increase in pituitary gland size by ≥3mm after cICI or 2) in the absence of baseline imaging for comparison, an interval decrease in gland size of ≥3mm on follow-up. These criteria were based on the clinical experience of the reporting radiologist.

Serial FDG-PET/CT scans were assessed by two independent nuclear medicine physicians (NMP) blinded to the clinical data. In the absence of established qualitative or semiquantitative criteria suggestive of hypophysitis on FDG PET/CT, for this study, a visibly perceptible increase in pituitary FDG uptake from baseline detected by both NMPs was considered positive. Using MIM software (MIM 6.7.11; MIM Software, Cleveland, OH), confirmatory semiquantitative analysis was performed using a 1cm spherical region of

interest over the pituitary fossa to assess maximum standardised uptake value (SUVmax) at baseline, post-treatment initiation and until resolution. Thyroid and adrenal SUVmax were also assessed.

Statistical methods

Baseline characteristics, prior treatment and adverse event profiles in patients with and without hypophysitis were compared using Fisher's exact, χ^2 and Wilcoxon rank sum tests. The interobserver agreement for the diagnosis of hypophysitis by FDG-PET/CT was assessed by the kappa statistic. Mann-Whitney U test and Kruskal-Wallis test were used for comparison of pituitary SUVmax and percentage increase in SUVmax among two or more groups, respectively. All biostatistical tests were performed by GraphPad Prism 8 (GraphPad Software, La Jolla, USA).

RESULTS

Participants

162 patients with a median age of 60 years (IQR 49-69) were included in the analysis. 49 (30%) had prior exposure to single agent ICI and 70 (43%) had received combination BRAF/MEK inhibition before cICI (Table 1).

Outcome data

Overall, 52/162 (32%) had evidence of hypophysitis, including 31 (19%) with documented clinical and biochemical changes and 21 (13%) suggested only by imaging (Figure 1). There was no difference in patient characteristics in patients with and without hypophysitis including prior treatment with single agent anti-CTLA-4 (6% versus 5% p=0.41). However,

primary thyroiditis diagnosed at any time during treatment was more common (37 versus 28% p<0.001) (Table 1).

Table 1. Clinical characteristics and immune toxicity

	Hypophysitis (n= 52)	**Non-Hypophysitis (n= 110)**	**p-value**
No. (%) Male	33 (63.5%)	80 (72.7%)	0.23
No. (%) Female	19 (36.5%)	30 (27.3%)	
Age, median (IQR), years	60.0 (50.2-69.3)	61.2 (48.6-68.0)	0.96
No. (%) Baseline ECOG			
0	45 (86.5%)	78 (70.9%)	0.17
1	5 (9.6%)	25 (22.7%)	
2	2 (3.8%)	6 (5.5%)	
3	0 (0.0%)	1 (0.9%)	
No. (%) Prior Exposure to ICI			
Ipilimumab	4 (7.70%)	5 (4.5%)	0.41
Nivolumab	0 (0.0%)	6 (5.5%)	0.09
Pembrolizumab	14 (27%)	26 (23.6%)	0.65
No. (%) Prior Exposure to Other Treatment			
Chemotherapy	3 (5.8%)	3 (2.7%)	0.34
BRAF/MEK Inhibition	24 (46.2%)	46 (41.8%)	0.60
No. (%) Prior Autoimmunity (where specified)			
≥1 autoimmune condition[a]	7 (13.5%)	12 (10.9%)	0.64
Combination immune checkpoint inhibition (cICI)			
Total number of cycles, median (IQR)[b]	6.5 (3.0-11.5)	4.0 (2.0-11.0)	0.10
Immune related adverse events			
No. (%) ≥1 irAE (other than hypophysitis)	47 (90.4%)	85 (77.3%)	0.05
No. (%) At least one grade 3/4 irAE	27 (51.9%)	42 (38.2%)	0.10
No. (%) Unplanned emergency or hospital presentation for irAE	37 (71.2%)	47 (42.7%)	<0.001
No. (%) Organ specific toxicity			
Dermatologic	26 (50.0%)	39 (35.5%)	0.078
Thyroiditis	19 (36.5%)	31 (28.2%)	<0.001
Hepatitis	19 (36.5%)	27 (24.5%)	0.11
Enteritis/colitis	18 (36.4%)	29 (26.4%)	0.28
Rheumatic	8 (15.4%)	11 (10.0%)	0.32
Pneumonitis	7 (13.5%)	8 (7.3%)	0.20
Nephritis	3 (5.8%)	1 (0.9%)	0.06
Myocarditis	1 (1.9%)	0 (0.0%)	0.14
Neurological	2 (3.8%)	4 (3.6%)	1.00
Other[c]	2 (3.8%)	2 (1.8%)	0.59

Table Footnotes:
Abbreviations: Eastern Cooperative Oncology Group Performance Status (ECOG), Immune checkpoint inhibition (ICI)
[a] Prior autoimmunity included rheumatoid arthritis, inflammatory bowel disease, psoriasis, vitiligo, Graves' disease, Hashimoto's disease, coeliac disease and type 1 diabetes.
[b] Patients received 4 cycles of combination ipilimumab plus nivolumab followed by single agent nivolumab
[c] Other toxicities included lymphadenitis, Haemolytic Anaemia, Panniculitis-like T-cell Lymphoma)

Documented Hypophysitis

There was variation in the clinical practice on the unit with some clinicians requesting cortisol levels every 3-4 weeks and others only in the event of clinical suspicion. In the 31 patients with a clinical diagnosis of hypophysitis, the median time to diagnosis was 9.6 weeks (range 0.7-40) and occurred after a median of 3 cycles (range 1-14). The majority (25/31) were diagnosed based on a low cortisol level after the patient reported new symptoms, most commonly, lethargy (n=18), headache (n=16) or anorexia/nausea (n=8). Visual disturbance was reported in 2 patients and adrenal crisis, weight loss, and delirium occurred as single events. Routine surveillance or imaging-initiated cortisol and thyroid function tests led to the diagnosis in an additional 6 patients (Figure 1). Available anterior pituitary hormone levels are outlined in Supplemental Tables 1 and 2.

Figure 1. Schema highlighting the methods of detection of hypophysitis in the entire cohort

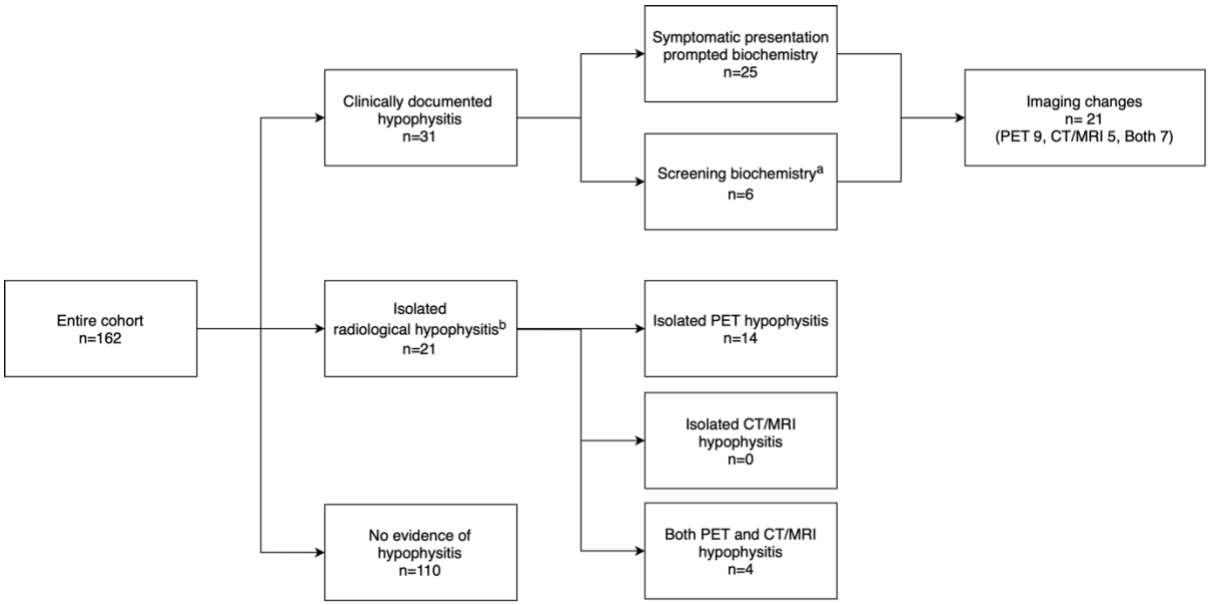


Figure legend:
Abbreviations: Positron Emission Tomography (PET), Computed Tomography (CT), Magnetic Resonance Imaging (MRI)
[a] Includes 3 patients where abnormal imaging prompted biochemical screening
[b] Patients with isolated radiological hypophysitis were detected retrospectively and did not have confirmatory pituitary hormones available.

Figure 2 outlines the precipitous drop seen in most patients from normal range cortisol to a median nadir cortisol level of 42 nmol/L (nadir cortisol range 3-102). Adrenocorticotropic hormone (ACTH) concentrations were measured in 18/31 patients, all of which were inappropriately low, consistent with pituitary-adrenal axis dysfunction. Mild, asymptomatic hyponatraemia was observed in 8 patients and occurred contemporaneously with cortisol deficiency (Supplemental Table 2). In all clinically diagnosed cases glucocorticoid replacement was commenced and subsequent withdrawal was not attempted.

Figure 2. Serial cortisol levels in patients with documented hypophysitis

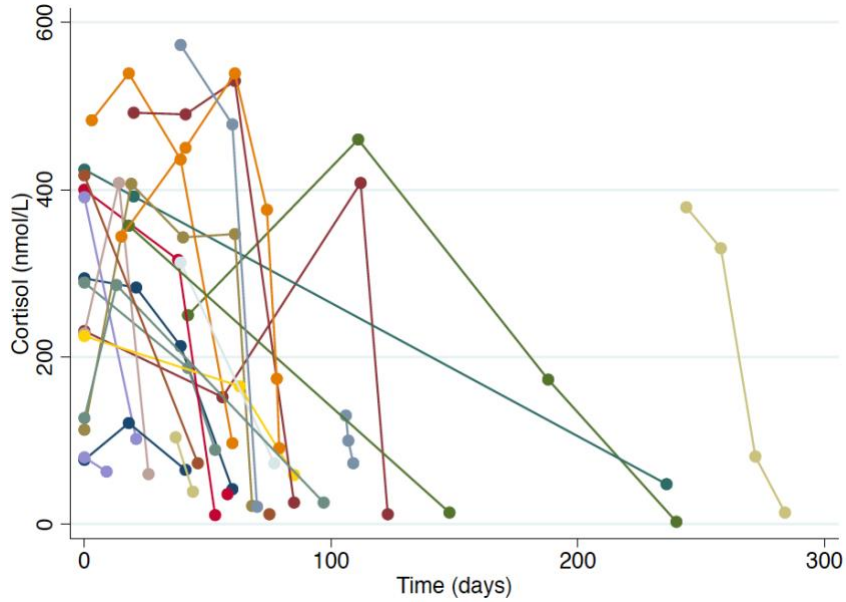


Figure Legend:
Each coloured line represents an individual patient, demonstrating the precipitous drop in cortisol level observed in patients where regular cortisol testing was performed.

Thyroid function tests were interpreted carefully and with a focus on delineating primary thyroiditis from thyroid dysfunction secondary to hypophysitis (Supplemental Information Item 2.0). Overall, 22% and 19% of patients diagnosed with hypophysitis developed permanent and transient pituitary-thyroid axis dysfunction, respectively. Longitudinal changes in thyroid function tests are outlined in further detail in Supplemental Table 1.

Complete gonadal axis assessment including luteinizing hormone (LH), follicule-stimulating hormone (FSH) and testosterone in males/oestradiol in females was available at hypophysitis diagnosis in 14/31 patients, 7 of whom had evidence of hypogonadism. Persistent hypogonadotropic hypogonadism was observed in 2/7 patients assessed at follow up (Supplemental Tables 1 and 2). Diabetes insipidus did not occur. Prolactin and growth hormone were not routinely assessed.

CT/MRI neuroimaging

CT or MRI brain imaging covering the pituitary fossa was available for retrospective evaluation in 100/162 patients with 73% assessable within 2 months of commencing cICI, 23% within 4 months and the remainder within 6 months (Supplemental Information Item 1.1). Reasons for a lack of post-treatment neuroimaging included short post-treatment survival and geographic/logistic limitations. Sagittal measurement of the gland size in the plane of the pituitary stalk was achievable in all scans. A definitive change in pituitary gland size ≥3mm occurred in 16/100 patients. In two of these baseline imaging was not performed, but sequential reduction in gland size by ≥3mm was observed within 6 months of follow-up imaging. All 16 patients had either a documented clinical diagnosis of hypophysitis (n=12) and/or FDG-PET/CT features consistent with hypophysitis (n=11). The 4 patients without a documented diagnosis were receiving long-term glucocorticoids for concomitant irAEs and did not undergo pituitary hormone evaluation within the period of study evaluation, but also had a positive FDG PET/CT.

Typical evolution of pituitary gland size in a patient with documented hypophysitis is demonstrated in Figure 3. The largest absolute gland size was 13mm, associated with a relative increase of 7mm from baseline (normal pituitary size is 9mm in females and 8mm in males). This was the only case where an enlarged gland abutted the optic chiasm. There were no cases of definite optic chiasm compression. Imaging changes preceded a clinical diagnosis of hypophysitis in 6/16 patients, by a median of 18 days (range 5-82 days.) Pituitary enlargement was universally transient. The mean final pituitary size was 5mm and was not different between patients with and without a documented diagnosis of hypophysitis.

Figure 3. Temporal changes in pituitary gland size and metabolic activity in a patient with documented clinical hypophysitis

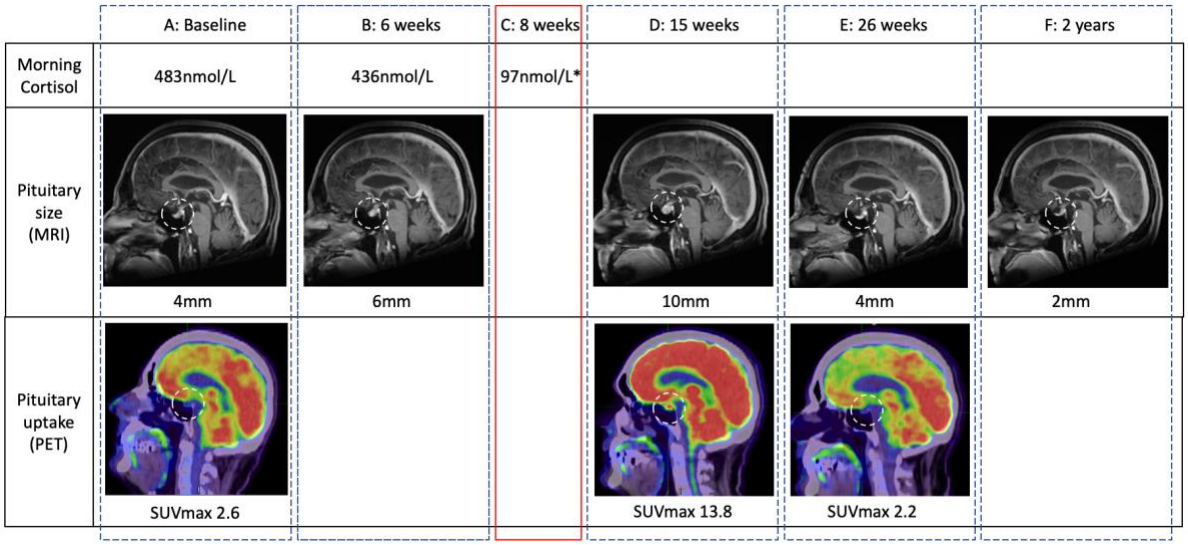


Figure legend:
The location of the pituitary fossa is indicated with a dotted white circle.
Abbreviations: Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET)
A: Normal baseline pituitary imaging and morning cortisol
B: MRI performed at 6 weeks for surveillance of existing brain metastases demonstrates a 2mm increase in pituitary size. Morning cortisol remains normal
C: At 8 weeks, the patient complained of headache and lethargy. Morning cortisol was subnormal and a clinical diagnosis of hypophysitis was made.
D: 7 weeks after symptomatic cortisol deficiency, pituitary size and metabolic activity are significantly increased from baseline
E and F: Follow up imaging demonstrates return to baseline pituitary size and avidity, and eventual reduction to sub-baseline size

Whole Body FDG-PET/CT

Baseline and post-treatment whole body FDG-PET/CT scans were undertaken in 134/162 patients. A visibly perceptible increase in pituitary FDG uptake suspicious for hypophysitis was noted by both observers in 36/134 patients at median 79 days from first treatment (range 20-126). There was high interobserver agreement between two NMPs, kappa 0.76 (95% confidence interval [CI] 0.64-0.88).

Typical evolution of pituitary SUVmax in FDG-PET/CT detected hypophysitis is demonstrated in Figure 3. Prior to immunotherapy, the median baseline pituitary SUVmax

for the cohort was 2.7 (IQR 2.5-3.1). In the 36 patients with FDG-PET/CT detected hypophysitis, the median SUVmax at diagnosis was 4.9 (IQR 3.9-5.6), with a corresponding increase of 68% (IQR 44-117.) Additional follow up scans undertaken as part of routine imaging were available in 30 of these 36 patients. Resolution of SUVmax to baseline metabolic activity occurred in all patients by 179 days (IQR 149-207) (Figure 4 and Supplemental Figure 1).

Figure 4. Temporal changes in pituitary SUVmax in the whole cohort

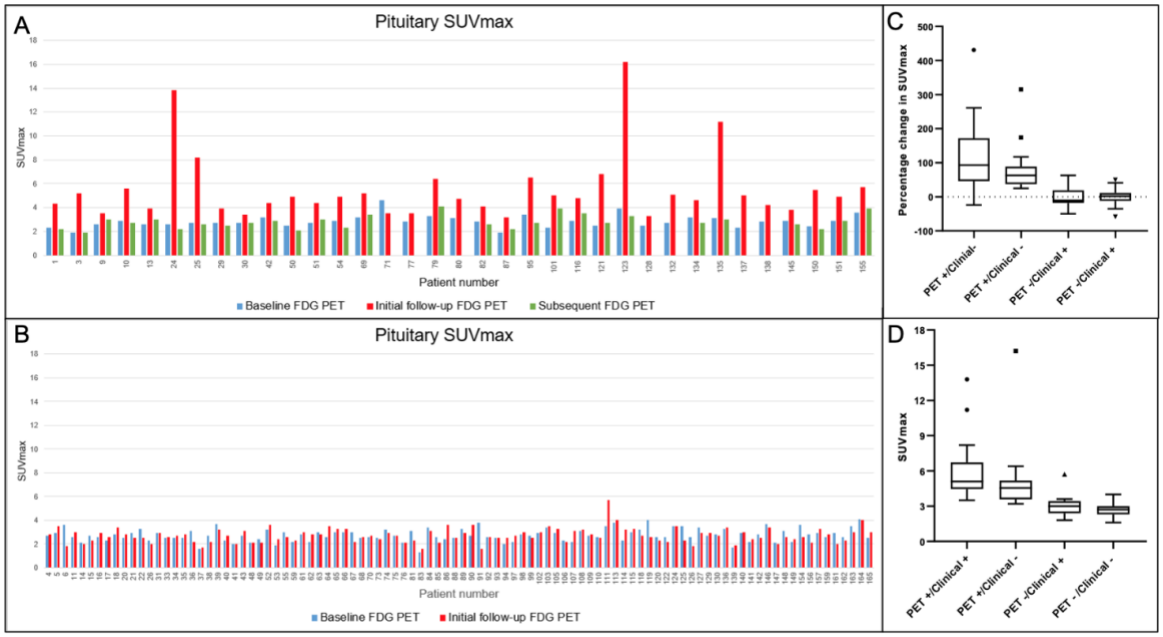


Figure legend:
Abbreviations: Maximum standardized uptake value (SUVmax), Positron Emission Tomography with Fluorodeoxyglucose (FDG PET)
A: Patients visually assessed as FDG PET-detected hypophysitis. Subsequent change in SUVmax is demonstrated in green bar if a further follow-up FDG PET was performed.
B: Patients without evidence of PET-detected hypophysitis.
C: Comparison of percentage change in SUVmax among different groups. Both demonstrate significant difference in pituitary uptake among groups (p value for both <0.0001).
D: Comparison of pituitary SUVmax on initial follow-up FDG PET among different groups.

Of 31 patients with a documented diagnosis of hypophysitis, 16 (52%) had FDG-PET/CT changes, which preceded the clinical diagnosis in 8 patients by a median of 22.5 days (range 5-182 days). Of the 20 patients with FDG-PET/CT changes without a confirmed clinical diagnosis, 18 were receiving long term glucocorticoids for another irAE

and pituitary function was not assessed, while the other two had normal cortisol levels but no other pituitary hormones measured. Of the 36 patients with FDG-PET/CT detected hypophysitis, contemporaneous MRI was available in 31 and was corroborative in 11, equivocal in 6 and normal in 14 (Supplemental Table 2).

Overt increased FDG uptake in the pituitary and thyroid gland was seen contemporaneously in 12 patients. Thyroid stimulating hormone (TSH) was above the upper limit of normal at the time of increased pituitary SUVmax in 7 patients (elevated TSH values ranged from 6.6-69.8 mIU/L, normal range 0.3-4.9). There were no cases of detectable increase in FDG uptake in the adrenal glands to suggest adrenalitis in this cohort.

DISCUSSION

Clinical and/or imaging evidence of hypophysitis was found in 32% of patients with advanced melanoma treated with cICI, which is the highest frequency reported to date. The incidence of clinically documented hypophysitis in our patients treated with cICI was higher than the published incidence in patients treated with cICI (19% versus 8-10.5%)[9, 69]. Regular cortisol monitoring was not required in the CheckMate 067 trial and our numbers likely reflect increased vigilance in monitoring for this toxicity. Importantly, a significant proportion of our cases were either detected or suspected on the basis of routine imaging. As such, temporal changes in pituitary size and FDG uptake by serial imaging may have a complementary role in facilitating the prompt diagnosis of immune-related hypophysitis. The majority of cases detected exclusively by imaging had been prescribed long-term glucocorticoids for concomitant irAEs, which a common clinical scenario during the first 6 months of treatment.

Historically, autoimmune hypophysitis was a rare diagnosis and usually made by exclusion of other causes of hypopituitarism or pituitary enlargement. With a higher index of suspicion after ICI, the accepted criteria for diagnosis relies on biochemical confirmation of anterior pituitary hormone deficiency in a symptomatic patient[30]. This study and published reports suggest that the clinical syndrome of hypophysitis after ICI may be mild, non-specific, or masked by glucocorticoids.

While ESMO guidelines suggest measurement of LH, FSH, testosterone (males) and oestrodiol (females) in all suspected cases of hypophysitis, evaluation of gonadotropic hormones may be unreliable due to acute or chronic illness suppressing the axis[85]. We observed that assessment of the gonadal axis was infrequently undertaken in this cohort, which may have resulted in undetected hypogonadism. It is notable that the incidence of hypogonadism was 50% (7/14) when it was assessed underpinning the need to evaluate the gonadal axis longitudinally in all patients with hypophysitis. Primary thyroiditis is common and must be considered when assessing the pituitary-thyroid axis. An elevated TSH differentiates primary from secondary hypothyroidism. If the patient is taking thyroxine for primary hypothyroidism, pituitary-thyroid dysfunction may be masked. Similarly, the pituitary-adrenal axis cannot be evaluated in the presence of supraphysiological doses of glucocorticoids for concomitant irAEs[86]. Therefore, the biochemical diagnosis is difficult to establish in this cohort and an incomplete pituitary hormone panel in many patients was a limitation of this study. These factors make pituitary imaging potentially very useful.

Traditionally, a dedicated pituitary MRI would be requested in a patient with suspected hypophysitis. While it is possible that subtle heterogeneity may not be appreciated without targeted pituitary imaging, we observed that the defining transient increase in pituitary size in

hypophysitis can be observed on routine brain imaging performed to screen for or monitor brain metastases. The temporal relationship between the size and appearance of the gland before and after ICI administration differentiates hypophysitis from alternate diagnoses. The two main findings - that 1) the absolute peak gland size at hypophysitis onset is often still within normal limits; and 2) the increase in size from baseline is transient - suggest that a false negative result could occur when the gland size is not compared to baseline or when there is a delay between hypophysitis onset and MRI imaging.

Reports of increased pituitary FDG avidity before and after diagnosis of a hypopituitary state have raised the possibility of FDG-PET playing a role in the diagnosis[83, 84]. In our cohort, a transient increase in metabolic activity in the pituitary gland was observed in 36 patients with 16 having a confirmed clinical diagnosis. Only in 2 cases was the enhanced FDG-PET uptake in the pituitary gland conveyed to the treating physician prompting an evaluation of the pituitary-adrenal axis. It is not known whether increased metabolic activity on FDG-PET reflects an early immuno-inflammatory response, or what effect supraphysiological doses of glucocorticoids may have on reversibility of pituitary cell destruction if administered prior to symptom onset. Only retrospective studies where glucocorticoids were administered after symptomatic cortisol deficiency are available, and did not demonstrate a benefit[50]. The findings of our study emphasize the added advantage of specific attention to reviewing longitudinal changes in pituitary size on MRI brain or increased FDG uptake in the pituitary in patients having FDG PET/CT for therapeutic monitoring of ICI therapy.

An elevated TSH at the time of the abnormal FDG-PET /CT scan was a potential confounding factor in 7 patients with an isolated increase in pituitary SUVmax. Thyrotrope hyperplasia and diffuse pituitary hypermetabolism of FDG in the hypothyroid state is well

described[87, 88]. It is feasible that increased pituitary SUVmax may have been caused by physiological TSH overproduction in response to primary hypothyroidism in these patients. Therefore, careful correlation of thyroid function tests, thyroid autoantibodies and other pituitary hormone levels are required in interpreting imaging findings.

Our findings prompt several recommendations for monitoring endocrinopathies during cICI therapy. Morning cortisol, TSH, T3 and T4 should be measured at each cycle for the first 4 cycles and every 4-8 weeks thereafter, or earlier in the presence of new onset headache, lethargy or malaise/anorexia. Radiologists and nuclear medicine physicians should assess the pituitary gland on routine imaging performed within the first 6 months after commencing cICI, looking for an increase in gland size or FDG uptake with respect to baseline. A finding of a low cortisol, secondary hypothyroidism, pituitary enlargement or increased pituitary SUVmax should prompt a full anterior pituitary hormone panel regardless of symptomatology. Hormone deficiencies in the adrenal, thyroid and gonadal axes should be treated with physiological replacement doses and cICI can be safely continued. In a glucocorticoid-treated patient with isolated imaging changes, a paired morning cortisol and ACTH should be measured on cessation of steroid treatment or once the dosage is as low as 5mg of prednisolone (or equivalent.) Patients requiring ongoing pituitary hormone replacement should be referred for Endocrinologist assessment, to evaluate for potential recovery of pituitary dysfunction and careful withdrawal of hormone replacement at follow up.

CONCLUSIONS

The combination of clinical diagnosis and imaging resulted in findings suggestive of hypophysitis in one third of patients with advanced melanoma treated with combination

ipilimumab and nivolumab. Importantly, we have shown that evaluation of routine structural assessment by CT/MRI and metabolic evaluation by FDG-PET/CT for specific changes in the pituitary gland may play a complementary role in the diagnosis of hypophysitis. The frequency of transient increases in pituitary size and metabolic activity following cICI in our study suggests regular biochemical monitoring for hypophysitis including adrenal, thyroid and gonadal hormone assessment is warranted. Routine testing may diagnose patients with a subclinical presentation who nevertheless have significant pituitary dysfunction and helps identify those whose diagnosis is being masked by glucocorticoid treatment for other irAEs.

A high index of suspicion, regular monitoring and a multimodality approach for timely diagnosis and management of hypophysitis are critical.

Online Only Supplemental Information

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Galligan, A, Iravani, A, Lasocki, A, Wallace, R, Weppler, A et al. The complementary role of routine cancer imaging in identifying clinically-occult hypophysitis after combination immune checkpoint inhibition.

Contents

1. Additional methods

1.1 Computed Tomography (CT) and Magnetic Resonance Imaging (MRI)

The frequency of structural neuroimaging depended on the clinical scenario for individual patients. Baseline brain imaging was comprised of CT brain in 17 patients and MRI brain in 98. Surveillance imaging of the brain with either CT or MRI generally occurred 2-4 monthly in patients with stage IV melanoma or more frequently if there were clinical symptoms warranting additional investigation.

73 patients had available imaging within the 2 months from commencing combined immune checkpoint inhibitors (cICI) (6 CT, 67 MRI). 23 patients had available imaging within 3-4 months from commencing cICI (5 CT, 18 MRI). The remaining patients had MRI imaging 5-6 months from cICI.

CT and MRI changes were reviewed by an independent radiologist blinded to clinical information. The outcome was considered equivocal if there was a change in size by <3mm or a new subjective bulkiness to the pituitary gland. Equivocal changes were classified as negative in the analysis but are described in Supplementary Table 2.

1.2 FDG-PET/CT

All studies were performed on an integrated PET/CT scanner including Biograph 16 (Siemens Medical Solutions, Erlangen, Germany), GE 690 and GE 710 (GE Healthcare, Milwaukee, WI), with routine cross-calibration three monthly. FDG-PET/CT was performed as per European Association of Nuclear Medicine Research Ltd. (EARL) initiative [89].

42 patients had available FDG-PET/CT imaging within 2 months from commencing cICI. 81 patients had FDG-PET/CT within 3-4 months from commencing cICI and the remaining had imaging 5-6 months from commencing cICI. The median time from commencement of cICI to the first PET scan was 76 days (range 18-225).

Two nuclear medicine physicians reviewed each scan independently of each other and were blinded to clinical information. Majority agreement was used to define FDG-PET/CT detected hypophysitis. If only one user noted an increase in pituitary SUVmax, the result was considered equivocal. Equivocal results were classified as negative in the analysis but are illustrated in Supplemental Table 2 and Supplemental Figure 1.

2. Thyroid function tests and their interpretation in patients with hypophysitis

Overt primary thyroid dysfunction is characterised by either a hyperthyroid state (high T3 and T4 with a suppressed TSH) or primary hypothyroidism (low T3 and T4 with an elevated TSH.) When evaluating a patient with suspected hypophysitis, the following considerations were made.

An elevated TSH in the setting of a low or low normal T4 indicates that the pituitary gland is able to mount an appropriate negative feedback response to a hypothyroid state, thus differentiating primary from secondary hypothyroidism. In the case of secondary hypothyroidism due to hypophysitis, the T4 is low because of insufficient production of TSH. In the hyperthyroid state, the TSH will be suppressed and thyrotrope deficiency cannot be excluded until resolution of thyrotoxicosis. If the patient is taking thyroxine for primary hypothyroidism, pituitary-thyroid dysfunction may be masked.

In the 31 patients with a clinical diagnosis of hypophysitis, 11 patients had evidence of primary thyroid dysfunction (presenting with thyrotoxicosis in 10 and primary hypothyroidism in 1) preceding or at the time of cortisol deficiency. Of these, thyroid function normalised in 4 patients, permanent secondary hypothyroidism developed in 3 patients, and in 3 patients permanent primary hypothyroidism evolved. The development of primary hypothyroidism as defined by a low T4 and high TSH is of interest in this cohort, as this is a definitive indicator of an intact pituitary-thyroid axis at follow up. There were 20 patients with no evidence of primary thyroid dysfunction at hypophysitis onset. 9/20 patients developed secondary hypothyroidism, which was transient in 5 patients and permanent in 4. Overall, long term thyroxine replacement was required in 7 (58%) of patients diagnosed with both thyroiditis and hypophysitis, and 4 (20%) of patients with hypophysitis only.

Supplemental Table 1. Adrenal, thyroid and pituitary function in the 31 patients with clinically documented hypophysitis

Adrenal Axis[a]	Hypocortisolism		28/31
	Not assessed due to concurrent glucocorticoid requirement		3/31
Thyroid Axis[b]	Primary thyroiditis before hypophysitis onset (8/31)[c]	Recovery of primary thyroiditis	3/8
		Permanent secondary hypothyroidism	2/8
		Permanent primary hypothyroidism	3/8
	Primary thyroiditis at hypophysitis onset (3/31)[d]	Transient primary thyroiditis	1/3
		Permanent secondary hypothyroidism	1/3
		Permanent primary hypothyroidism	1/3
	No primary thyroid dysfunction (20/31)	Persistent euthyroidism	7/20
		Transient secondary hypothyroidism	5/20
		Permanent secondary hypothyroidism	4/20
		Not assessed	4/20
Gonadal Axis	Permanent hypogonadotropic hypogonadism		2/31
	Transient hypogonadotropic hypogonadism		7/31
	Not assessed		22/31

Figure Legend:
[a] A clinical diagnosis of hypophysitis was made by the clinician based on symptoms, anterior hormone levels and in some cases, pituitary imaging. In the three people receiving glucocorticoids, the diagnosis was made based on results of thyroid and gonadal function and imaging changes.
[b] To describe the evolution of thyroid function, patients were grouped according to those with prior thyroiditis, concomitant hypophysitis and thyroiditis, and those with no evidence of thyroiditis
[c] 8 patients had initially presented with transient thyrotoxicosis at a median of 100 days prior to hypophysitis onset. 3 demonstrated permanent recovery of the thyroid gland, 2 evolved into secondary hypothyroidism and 3 patients had persistent primary hypothyroidism. The persistently elevated TSH indicated an intact pituitary-thyroid axis
[d] 2 patients were clearly thyrotoxic at the time of acute cortisol deficiency, one demonstrated recovery of long-term thyroid function and one evolved into permanent secondary hypothyroidism.1 patient had primary hypothyroidism at the time of acute cortisol deficiency and at follow-up. The persistently elevated TSH indicated an intact pituitary-thyroid axis throughout the episode of hypophysitis.

Supplemental Table 2: Clinical, biochemical and imaging features of patients with a clinical or radiological diagnosis of hypophysitis.

	Hypophysitis diagnosis	**Symptoms[a]**	**Glucocorticoid use/indication**	**Pituitary hormones[b]**	**CT/MRI findings[c]**	**PET findings[d]**
1	Isolated PET findings	None reported	Yes/ Pneumonitis, enteritis	Cortisol 58 ACTH 2.2 (while receiving glucocorticoid) TSH 0.04, T4 10.5 (transient, recovered within 1 month Other pituitary hormones not available	Normal MRI	PET hypophysitis
2	Isolated PET findings	None reported	Yes/ Thyroiditis, hepatitis, enteritis, pancreatitis	TSH 25.9, T4 <5.10 (pituitary-thyroid axis intact, required thyroxine) Other pituitary hormones not available	Normal MRI	PET hypophysitis
3	Isolated equivocal MRI and equivocal PET findings	None reported	Yes/ Hepatitis	Random cortisol 248 after ceasing prednisolone Other pituitary hormones not available	No increase in gland size but subjectively "bulky" appearance	Equivocal PET (1 user only)
4	Symptoms prompted biochemistry	Headache Lethargy Anorexia Blurred vision	No	Adrenal and thyroid axes involved: Cortisol 42 ACTH not available Na 121 TSH 0.1, T4 8.2	Normal MRI at time of biochemical diagnosis	Normal PET
5	Isolated PET findings	Abdominal pain at the time of low cortisol	Yes/ Nephritis	Cortisol 25 attributed to adrenal metastases, just prior to commencing glucocorticoids Cortisol 365 2 months after cessation of glucocorticoids	Normal MRI	PET hypophysitis
6	Symptoms prompted biochemistry	Headache Lethargy Blurred vision	No *note prior thyroiditis	Adrenal and thyroid axis involved: Cortisol 12, ACTH <1.0, Na 137 TSH 0.32, T4 11.5 T3 1.9 LH 11.6, FSH 52.8, Oestrodiol 49 (appropriate post-menopausal range)	MRI hypophysitis	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
7	Isolated PET findings Equivocal MRI findings	None reported	No	1 out of 3 suboptimal random cortisol levels (237, 127, 320) in the 6 months post imaging Transient changes suggestive of thyroid axis involvement (TSH 0.14, T4 11.6) Other pituitary hormones not available	No increase in gland size but subjectively "bulky" appearance and subsequent 2mm size reduction	PET hypophysitis
8	Isolated equivocal MRI changes	Hypotension	No	1 out of 2 suboptimal morning cortisol levels (255, 172) TSH 1.58 T4 13.1 T3 5.2 Other pituitary hormones not available	Equivocal MRI (1-2mm increase)	Normal PET
9	Isolated equivocal PET changes	None reported	No	Random cortisol levels 252, 200, 215 TSH 1.63 T4 11.2 T3 4.1	Normal MRI	Equivocal PET (1 user only)
10	Isolated equivocal PET findings	Fatigue and postural hypotension while weaning glucocorticoid	Yes/ Colitis *note prior thyroiditis	ACTH 3.5, cortisol 191 (on glucocorticoids) TSH 1.02, T4 10.0, T3 3.5 Random cortisol 179 after glucocorticoids ceased Other pituitary hormones not available	Normal MRI	Equivocal PET (1 user only)
11	Symptoms prompted biochemistry	Lethargy	No	Adrenal axis only: Cortisol 12, ACTH 1.9, Na 136 On thyroxine at presentation, euthyroid. LH/FSH normal	MRI not available	Normal PET
12	Symptoms prompted biochemistry	Headache Lethargy	No	Adrenal and thyroid axes involved: Cortisol 97, ACTH not available, Na 140 TSH 3.5, T4 6 (required thyroxine)	MRI hypophysitis	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
				Normal testosterone at follow up		
13	Symptoms prompted biochemistry	Headache	No	Adrenal, thyroid and gonadal axes involved: Cortisol 89 ACTH <3 Na 135 TSH 1.43 T4 <5.10 Testosterone 0.8	MRI hypophysitis	PET hypophysitis
14	Symptoms prompted biochemistry	Headache Lethargy	No	Adrenal and thyroid axes involved: Cortisol 11, ACTH 1.8, Na 136 TSH 0.09, T4 7.7 Testosterone 25.3 2 months later	MRI hypophysitis	FDG-PET not available
15	Isolated MRI and PET findings	None reported	Yes/ Hepatitis	Cortisol 286 (on glucocorticoid) Transient evidence secondary hypothyroidism (TSH 0.19 T4 9.60) Other pituitary hormones not available	MRI hypophysitis	PET hypophysitis
16	Isolated PET findings	None reported	Yes/ Arthritis, sicca syndrome	Random cortisol 132 while on prednisolone 10mg TSH 0.78 at the time of PET, and persistently normal after Other pituitary hormones not available	MRI not available	PET hypophysitis
17	Low cortisol on safety bloods	Lethargy	Yes/ Colitis	Adrenal axis only**:** Cortisol 102, Na 143 (just before commencing glucocorticoids) Other pituitary hormones not available at diagnosis Normal TFTs at follow up (TSH 0.88, T4 13.8)	Normal MRI	PET hypophysitis
18	Symptoms prompted biochemistry	Lethargy	No	Not available (managed externally)	MRI not available	Normal PET

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
19	Symptoms prompted biochemistry	Lethargy	Yes/ Pneumonitis	Adrenal axis only: Cortisol 12 after steroids ceased. ACTH not available Primary thyroiditis treated with thyroxine Normal gonadotropes at follow up	Normal MRI	PET hypophysitis
20	Isolated PET findings	None reported	Yes/ Pneumonitis, thyroiditis	TSH 31.3, T4 9.7, T3 2.5 (pituitary thyroid axis intact) Other pituitary hormones not available	Normal MRI	PET hypophysitis
21	Isolated PET findings Later unable to be weaned from steroids due to low cortisol	None reported	Yes/ Arthritis	Cortisol 62, TSH 1.29 T4 11 at the time of abnormal PET (was receiving prednisolone). No gonadotropes measured. 6 months later after ceasing prednisolone, cortisol 101 and ACTH <0.3, attributed to adrenal axis suppression. TFT remained normal (TSH 2.05 T4 12.7)	Equivocal MRI (1-2mm increase)	PET hypophysitis
22	Low cortisol on safety bloods	Lethargy Dehydration	No *note prior thyroiditis	Adrenal axis only: Cortisol levels 130, 100, 73 ACTH not available, Na 141 Transient primary thyroiditis (TSH <0.01 T4 37.4), no gonadotropes	Normal MRI	Normal PET
23	Abnormal MRI reported to clinicians, diagnosis made without biochemistry	Headache	Yes/ Colitis	Na 134 TSH 0.67 and remained normal Other pituitary hormones not available	MRI hypophysitis	Equivocal PET (1 user only)
24	Symptoms prompted biochemistry	Lethargy	No	Adrenal axis only: Cortisol 136, then <20 Na 136 ACTH 2.1, TSH 8.93 (intact	MRI not available	Normal PET

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
				pituitary thyroid axis), LH/FSH normal no testosterone		
25	Isolated equivocal PET changes	None reported	No	Morning cortisol 301 1 month prior to abnormal PET Transient secondary hypothyroidism (TSH 0.39 T4 8.9)	MRI not available	Equivocal PET (1 user only)
26	Isolated PET findings	None reported	Yes/ CNS metastases, Thyroiditis, rash	Random cortisol 183-366 in between glucocorticoid courses TSH 9.34, T4 6.2 (Pituitary thyroid axis intact)	Normal MRI	PET hypophysitis
27	Symptoms prompted biochemistry	Lethargy Anorexia	No	Adrenal axis only: Cortisol 22 ACTH <1.0, Na 133 TSH 0.10, T4 14.9 6 months later developed primary hypothyroidism (TSH 14.4, T4 9.7) indicating intact pituitary thyroid axis LH/FSH low at follow up	MRI hypophysitis (decrease in gland size >3mm)	PET hypophysitis
28	Abnormal PET prompted biochemistry	None reported	Yes/ Colitis	Adrenal, thyroid and gonadal axes: On steroids at diagnosis, unable to be weaned. Secondary hypothyroidism (TSH 0.42 T3 2.3 T4 10.7) low LH and FSH	Equivocal MRI (decrease in gland size 2mm)	PET hypophysitis
29	Isolated PET findings	None reported	Yes/ Hepatitis	At the time of abnormal PET, TSH 0.04, T4 12.7 T3 3.1 After cessation of glucocorticoid: cortisol 332, ACTH 7 TSH 1.13 T4 13.9	Normal MRI	PET hypophysitis
30	Isolated MRI and PET findings Later unable to be weaned from	None reported	Yes/ Hepatitis	Pre-dose cortisol 21 while on 5mg prednisolone TSH 1.99 T4 6.7	MRI hypophysitis	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
	steroids due to low cortisol					
31	Isolated PET findings	None reported	Yes/ CNS disease	Cortisol 15 (recently prescribed dexamethasone, unclear if taking at the time of the test) TSH 1.17, T3 2.1, T4 11 (Suspicious for thyroid axis involvement)	Normal MRI	PET hypophysitis
32	Symptoms prompted biochemistry	Headache	No	Adrenal, thyroid and gonadal axes: Cortisol 60, ACTH 22, TSH 0.86, T4 10.6 low LH	MRI not available	PET hypophysitis
33	Isolated equivocal PET changes	None reported	Yes/ Arthritis	At the time of the abnormal PET, cortisol 47 (on prednisolone) TSH 0.68, T4 12.5 After prednisolone ceased, Morning cortisol 561 TFT remained normal	MRI not available	Equivocal PET (1 user only)
34	Isolated PET findings	None reported	No	At the time of abnormal PET, morning cortisol 209, TSH 3.0 TFT and cortisol normal 2 months later. Other pituitary hormones not available	Normal MRI	PET hypophysitis
35	Symptoms prompted biochemistry	Lethargy	No	Adrenal and thyroid axes: Cortisol 59 ACTH not available, Na 136 TSH 1.18, T4 8.80 T3 1.9	MRI images inadequate but pituitary appears to decrease in size	Equivocal PET (1 user only)
36	Isolated equivocal PET changes	Lethargy	No	Cortisol 604, 334 TSH 1.63 T4 11 Other pituitary hormones not available	MRI not available	Equivocal PET (1 user only)
37	Symptoms prompted biochemistry	Lethargy Nausea	No	Adrenal axis only: Cortisol 14, ACTH not available,	MRI not available	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
				Na 130 TSH 0.84, T4 9.50 (persistent euthyroidism)		
38	Isolated PET findings Later unable to be weaned from steroids due to low cortisol	None reported	Yes/ CNS disease, thyroiditis, rash/SJS	Pre-dose cortisol level 13 while on 5mg prednisolone TSH 68, T4 5 (pituitary thyroid axis intact)	Normal MRI	PET hypophysitis
39	Isolated equivocal PET changes	None reported	No	Cortisol 536, TSH 2.24 T4 10.7 3 2.3	Normal MRI	Equivocal PET (1 user only)
40	Symptoms prompted biochemistry	Delerium	No	Adrenal, thyroid and gonadal axes: Cortisol 73, ACTH not available TSH 0.22, T4 8.6 LH/FSH low	MRI not available	Equivocal PET (1 user only)
41	Isolated equivocal PET changes	None reported	No	Random cortisol 217, TSH 0.5 T4 14.8	Normal MRI	Equivocal PET (1 user only)
42	Isolated equivocal MRI	Neurological changes attributed to CNS disease	Yes/ CNS disease, hepatitis	TSH 0.08 T4 12.1 T3 3.1 Other pituitary hormones not available	Small increase in gland size with “bulky” appearance	Normal PET
43	Isolated equivocal MRI before cICI (while taking PD-1 monotherapy)	None reported	Yes/ Arthritis	Hyponatraemia Cortisol 40, unclear if on glucocorticoids	Small increase in gland size with “bulky” appearance	Normal PET 3 months after abnormal MRI
44	Low cortisol on safety bloods	Lethargy Anorexia	No *Prior thyroiditis	Adrenal axis only: Cortisol 65 ACTH 0.6, Na 135 TSH 0.01 T4 14 (recovering thyroiditis) High TSH at follow up indicates intact pituitary thyroid axis. LH/FSH normal at follow up.	Normal MRI 2 months after presentation	Equivocal PET (1 user only)
45	Isolated equivocal PET changes Later unable to be weaned from steroids due to low cortisol	Headache (attributed to CNS disease)	Yes/ CNS disease, colitis, thyroiditis, encephalomyelitis	Cortisol 19 after ceasing steroids ACTH not available TSH 35, T4 2.6 (intact pituitary thyroid axis) Gonadotropes not available	Normal MRI	Equivocal PET (1 user only)
46	Isolated PET findings	Headache and nausea (attributed to CNS disease)	Yes/ CNS disease, pneumonitis, thyroiditis	Cortisol 125 after ceasing glucocorticoids	Normal MRI	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
				TFT normal at time of abnormal PET. A month later, transient secondary hypothyroidism TSH 0.26 T4 8.6		
47	Isolated equivocal PET changes	None reported	No	Cortisol 448, no ACTH TSH 55 T4 <5.1 (intact pituitary-thyroid axis) Gonadotropes not available	Normal MRI	Equivocal PET (1 user only)
48	Symptoms prompted biochemistry	Headache	No	Adrenal axis only: Cortisol levels 73 and 26, ACTH 5.2 Na 142 TSH 0.51, T4 11, T3 3.4 LH/FSH normal Euthyroid at follow up	No increase in gland size but subjectively "bulky" appearance	PET hypophysitis
49	Isolated MRI and PET findings	Headache (attributed to CNS disease)	Yes/ Hepatitis	One undetectable cortisol (<14) just prior to starting glucocorticoids TSH 35, T4 4 (Primary hypothyroidism) at the time of abnormal scan	MRI hypophysitis	PET hypophysitis
50	Isolated PET findings	None reported	Yes/ Colitis	Cortisol 223, ACTH 2.3 (on prednisolone) TSH 0.3, T4 12.10 (not repeated)	Normal MRI	PET hypophysitis
51	Isolated PET findings	None reported	Yes/ CNS disease, nephritis	None available	Normal MRI	PET hypophysitis
52	Symptoms prompted biochemistry	Headache Malaise	No	Adrenal and gonadal axes: Cortisol 14 ACTH<1.0, Na 119 TSH 0.68 T4 15.3 FSH 3.9 Testosterone 7.1	No increase in gland size but subjectively "bulky" appearance	PET hypophysitis
53	Abnormal PET and MRI prompted biochemistry	Lethargy Anorexia	No	Adrenal, thyroid and gonadal axes: Cortisol 91, ACTH not available, Na 136 TSH 0.3, T4 8.7 Hypogonadism at follow up.	MRI hypophysitis	PET hypophysitis
54	Symptoms prompted biochemistry	Headache Dizziness	Yes Vitiligo, pancreatitis, colitis	Not available (offsite)	MRI hypophysitis	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
55	Low cortisol on safety bloods	None reported	No	Adrenal axis only: Cortisol 26 no ACTH, Na 142 TSH 2.28 T4 10.7	MRI not available	PET hypophysitis
56	Symptoms prompted biochemistry	Lethargy	No	Adrenal and gonadal axes: Cortisol 22 ACTH 4.5, Na 130, testosterone 4.2 TSH 8.85, T4 <5.10 transient low testosterone, permanent primary hypothyroidism required thyroxine	MRI hypophysitis	Normal PET
57	Isolated abnormal MRI and PET Later unable to be weaned from steroids due to low cortisol	None reported	Yes Pneumonitis, thyroiditis	Pre-dose cortisol 14 while on prednisolone 10mg. TSH 5.53, T4 <5.10 (intact pituitary thyroid axis), Appropriate LH and FSH for post-menopausal state	MRI hypophysitis	PET hypophysitis
58	Symptoms prompted biochemistry	Lethargy	Ceased a month prior to diagnosis	Adrenal axis only: Cortisol 63 ACTH 5.8 Na 133 Other pituitary hormones not available	MRI not available	Normal PET
59	Symptoms prompted biochemistry	Headache	No	Adrenal and thyroid axes: Cortisol 39, ACTH <0.7, TSH 0.10 T4 15.8 (transient secondary hypothyroidism) Normal LH/FSH Subsequent high TSH indicated recovery of pituitary thyroid axis	Normal MRI	Normal PET
60	Symptoms prompted biochemistry	Headache Nausea	No	Adrenal axis only: Cortisol 73 ACTH 4.5 TSH 0.49 T4 14.2, T3 4.5 (Transient low TSH) no gonadotrophes Normal testosterone (23.9) at follow up	No increase in gland size but subjectively "bulky" appearance	PET hypophysitis

	Hypophysitis diagnosis	Symptoms[a]	Glucocorticoid use/indication	Pituitary hormones[b]	CT/MRI findings[c]	PET findings[d]
61	Symptoms prompted biochemistry	Headache	No	Adrenal, thyroid and gonadal axes: Cortisol 21, no ACTH, Na 132 TSH 0.2, T4 11.60 Low FSH and LH for menopausal state	MRI hypophysitis	PET hypophysitis
62	Isolated PET findings	None reported	Yes, CNS disease, colitis, thyroiditis	Cortisol 204 just prior to commencing glucocorticoids TSH 30, T4 6 (intact pituitary thyroid axis)	MRI not available	PET hypophysitis
63	Safety bloods demonstrated secondary hypothyroidism	None reported	Yes/ Colitis, hepatitis	Thyroid axis only: TSH 0.2 T4 10.3 T3 3.8 **(**Permanent secondary hypothyroidism)	MRI hypophysitis	Normal PET 3 weeks before presentation
64	Symptoms prompted confirmatory biochemistry	Headache	Yes/ Hepatitis, autoimmune haemolytic anaemia	Adrenal axis only: Cortisol 114 just prior to starting glucocorticoids Na 142 Other pituitary hormones not available Euthyroid at follow up (TSH 2.02, T4 12)	MRI hypophysitis	Normal PET

Figure legend:
Abbreviations: Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), adrenocorticotripic hormone (ACTH), Sodium (Na), thyroid stimulating hormone (TSH), tetraiodothyronine (T4), triiodothyronine (T3), leutenizing hormone (LH), follicle stimulating hormone (FSH)

[a] Symptoms attributed to hypophysitis or reported at the time of abnormal imaging and not explained by a known alternate cause
[b] Pituitary hormones and biochemistry units and reference ranges: Cortisol nmol/L (morning >250, random >200), ACTH ng/L (7.2-63.3), Na mmol/L (135-145), TSH mU/L (0.35-4.94), T4 pmol/L (9.0-19), T3 pmol/L (2.6-5.7), Testosterone (males) nmol/L (8.3-30.2), LH and FSH are described as low, high or appropriate as adjudicated by an endocrinologist, based on the sex, age and gonadal status
[c] CT/MRI criterion for hypophysitis was a change in pituitary size >/= 3mm after combination immune checkpoint inhibition. Features including subjective bulkiness or a change in pituitary size <3mm are illustrated in this table as equivocal and were classified as negative (no MRI detected hypophysitis) in the analysis
[d] Majority agreement was used to define PET hypophysitis. If only one user detected an increase in pituitary uptake, the findings are illustrated as equivocal in this table and classified as negative (no PET detected hypophysitis) in the analysis

Supplemental Figure 1. Pituitary SUVmax and percentage change at first follow up

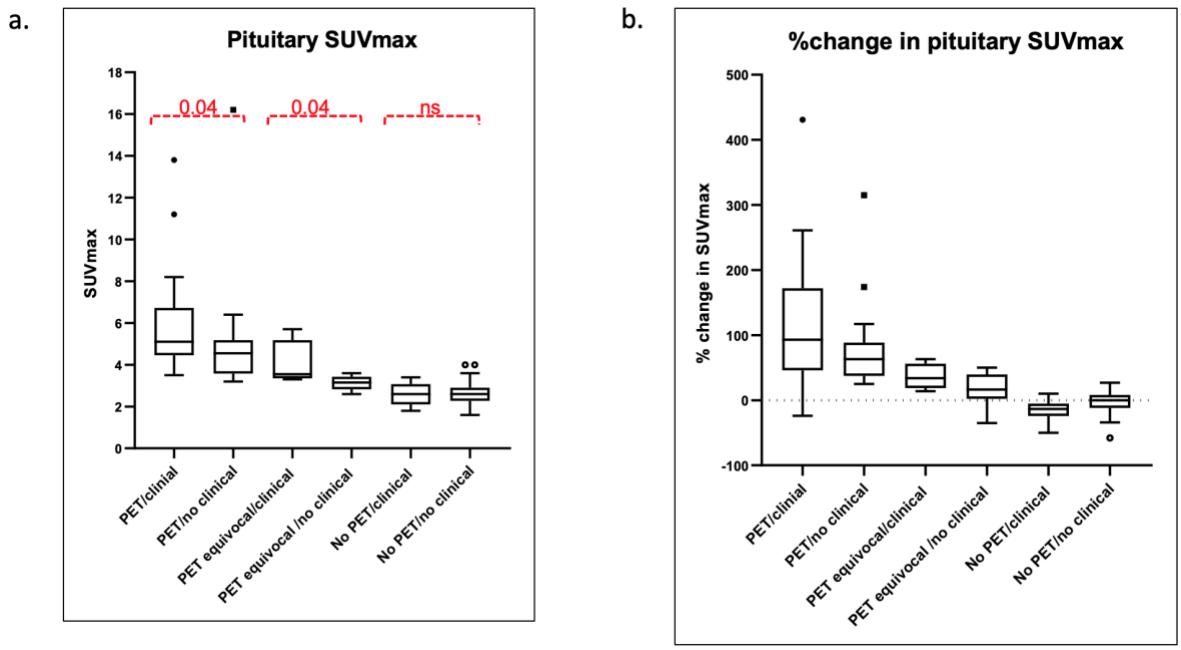


Figure Legend:
Abbreviations: Maximum standardised uptake value (SUVmax), Positron Emission Tomography (PET)

[a] Median SUVmax is presented among six groups depending on the PET findings: two users detect increased uptake (PET hypophysitis), one user detects increased uptake (PET equivocal) or both users agree no increased uptake (no PET hypophysitis) and the presence or absence of a documented clinical diagnosis. The median SUVmax among the six groups was significantly different ($p<0.0001$)
[b] %change in pituitary SUVmax with respect to baseline is presented among the same six groups and was significantly different between the groups ($p <0.0001$)

3.3 Unpublished survival data omitted from Chapter 3.2

Introduction

Potential correlation between immune toxicity and tumour response is at the forefront of many clinician-to-patient discussions. It has, for example, been shown that rheumatological irAEs seem to be associated with an increased objective response rate despite frequent symptomatic use of steroids[22]. To date, hypophysitis has not been strongly correlated with survival outcomes after ICI.

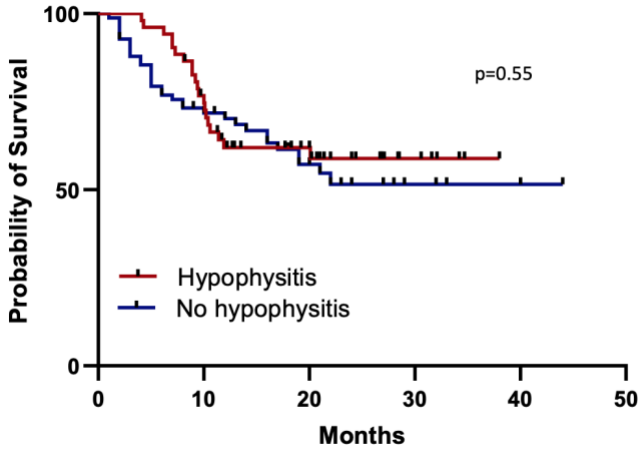
Methods

FDG-PET/CT response was assessed by PET response criteria in solid tumors (PERCIST) and grouped to objective response (complete or partial metabolic response) or no response (stable or progressive metabolic disease)[90]. Overall survival (OS) of patients with or without hypophysitis was compared by Log-rank test and depicted by the Kaplan-Meier method.

Correlation of hypophysitis and oncologic outcome

At median follow-up of 21 months, the median overall survival (OS) of the patients with and without hypophysitis was not reached with no significant difference between the groups, (HR 0.84, 95% CI 0.49-1.46, p=0.55) (Figure 1). In a subgroup of 108 patients with PERCIST measurable disease on FDG-PET, although the objective response was numerically higher in patients with hypophysitis compared to those without hypophysitis, the difference did not reach statistical significance (OR 41% and 33%, respectively, p=0.4).

Figure 1. Overall survival in patients with and without cICI related hypophysiti



Conclusion

Our study was not able to determine an association between hypophysitis and tumour response or OS, which may be due to limited power of the study and insufficient numbers.

3.4 Recommendations for the radiological diagnosis of hypophysitis

Introduction

Chapter 3.2 closely defined the radiological features of hypophysitis after cICI. In collaboration with colleagues from the Department of Cancer Imaging, we then provided an illustrative review with recommendations for the recognition of hypophysitis as opposed to other pituitary pathologies in a pictorial essay titled "The imaging of immunotherapy-related hypophysitis and other pituitary lesions in oncology patients" (Appendix 3). A summary of the findings and recommendations is presented below.

Key imaging features of hypophysitis

After careful review of the published literature and our own dataset, we describe the key imaging characteristics of ICI related hypophysitis, namely diffuse but modest pituitary enlargement which is universally transient. On MRI, the pituitary enhances homogeneously, and pituitary stalk thickening is observed in some but not all cases[91]. These classic radiographic features may be sought at the time of diagnosis or seen incidentally in patients undergoing brain imaging for tumour surveillance.

Pituitary size can be accurately assessed with both CT and MRI and the absolute size should be interpreted in the clinical context. In ICI related hypophysitis, the peak gland size is often within normal range but the relative change in size from baseline is a critical component of the diagnosis. The final gland size may be smaller than the baseline size suggesting destruction of pituitary tissue. A reduction in gland size from earlier to later scans while receiving ICI is considered suspicious if baseline imaging was not performed prior to treatment commencement. If the abnormality can be observed on baseline imaging prior to ICI treatment, hypophysitis is unlikely to be the cause.

The normal pituitary gland does not demonstrate increased FDG uptake above background activity, hence diffusely increased uptake is suggestive of a pituitary abnormality. FDG-PET cannot differentiate hypophysitis from other pathologies (see below) on a single scan, but like in CT and MRI, the temporal evolution of new and transient FDG uptake with respect to baseline is the defining feature.

Imaging features suggestive of an alternate diagnosis

Pituitary adenomas are relatively common, with imaging and autopsy population studies suggesting a prevalence of 17%. Microadenomas are small lesions <10mm in size that are unlikely to change significantly over time. Pituitary macroadenomas may be large enough to obscure the normal pituitary gland on imaging, cause a heterogenous appearance and may compress or invade local structures such as the cavernous sinus or optic chiasm. Sellar expansion is a sign of chronicity often seen with existing macroadenomas[92]. Stability of a lesion existing at baseline and throughout treatment with immunotherapy suggests a diagnosis of an existing pituitary adenoma or Rathke's cleft cyst, which may or may not demonstrate increased uptake of FDG on PET.

Pituitary metastases are rare, accounting for <0.5% of intracranial metastases[93]. Metastatic lesions generally have a heterogenous appearance and enhance less than the normal pituitary tissue which may be seen adjacent to the metastasis. Like other tumour deposits, these lesions can be aggressive. Invasion through the sellar floor, into the cavernous sinus or optic chiasm are suspicious for progressive disease. Consideration of the evolution of other intracranial tumour deposits is critical, as a pituitary metastasis should progress or respond to treatment in line with the other metastases.

Recommendations

1. Given the high incidence of hypophysitis, the pituitary size and appearance should be reviewed on routine imaging performed within the first 6 months of ICI
2. If the gland size measures ≥7mm or appears subjectively bulky, the current study should be compared to baseline imaging looking for a change in gland size
3. Recognition of an interval increase in gland size should prompt longitudinal monitoring of anterior pituitary hormones to identify evolving gland dysfunction
4. A gland size >2cm, pituitary stalk deviation, invasion into surrounding structures or the presence of a discrete lesion adjacent to normal pituitary tissue should prompt consideration of an alternative cause

3.5 Conclusion

The gold standard for the diagnosis of hypophysitis historically relied on a suspicious clinical presentation followed by a confirmatory panel of anterior pituitary hormones. My findings suggest that the clinical syndrome of hypophysitis after ICI may be mild, non-specific, or masked by concurrent glucocorticoid use. In the real-world setting, one-off biochemical pituitary hormone assessment in the unwell oncology patient is fraught with challenges. For instance, evaluation of gonadotropic hormones may be unreliable due to acute or chronic illness suppression of the axis[85]. Primary thyroid dysfunction is common, and the pituitary-thyroid axis evaluation cannot be relied on in the thyrotoxic patient or in the presence of thyroxine. The pituitary adrenal-axis cannot be evaluated in the presence of supraphysiological doses of glucocorticoids for concomitant irAEs[86]. In light of the limitations described, the diagnosis may be difficult to establish, and a multimodality approach becomes critically important. This involves both careful review of pituitary appearance on available neuroimaging and repeated longitudinal evaluation of pituitary hormones over time, both of which may identify evolving pituitary dysfunction before overt symptom onset. Once diagnosed with thyroid, adrenal or gonadal axis involvement, patients should be managed with physiological replacement of the deficient hormones. Referral to an endocrine clinic is appropriate, for careful titration to avoid over- or undertreatment and for consideration of treatment withdrawal to assess for gland recovery.

Chapter 4. Immune checkpoint inhibitor related thyroiditis

4.1 Introduction

Thyroiditis is a common irAE with both CTLA4 and PD1/PD-L1 inhibition. Blockade of the PD-1/PD-L1 pathway is associated with an incidence of 8-25% while CTLA-4 inhibition is associated with a slighter lower incidence of 6%[4, 94]. Several case series have described the timing and kinetics of ICI related thyroiditis[62]. There is significant heterogeneity in the form of dysthyroidism reported, including transient thyrotoxicosis, subclinical hypothyroidism and overt hypothyroidism. I hypothesised that these are stages of the same disease process, whereby immune thyroiditis causes release of preformed thyroid hormone after which destruction of the gland can lead to permanent hypothyroidism. In this chapter, I review the natural history of biochemical changes in thyroid hormone levels in a cohort of patients monitored very closely for thyroid dysfunction. Nuclear medicine studies, particularly technetium thyroid scans are particularly useful in the differentiation of thyroiditis from Graves' disease or autonomous nodules in the general clinic, however these scans are rarely performed in patients receiving immunotherapy. FDG-PET/CT imaging, however, is routinely utilised within the first 3-6 months of treatment for the evaluation of tumour response. We noticed the incidental finding of increased FDG activity in the thyroid was occurring with increasing frequency, however the relationship between these changes and clinical disease had not been evaluated. As such, we performed the first diagnostic accuracy study with an interobserver agreement for the role of FDG-PET/CT in the diagnosis of thyroiditis.

4.2 Diagnostic accuracy of FDG-PET/CT in the detection of thyroiditis on routine imaging after combination immune checkpoint inhibitors

Manuscript prepared for publication and re-titled: “Increased thyroidal activity on routine FDG-PET/CT after combination immune checkpoint inhibition: temporal associations with clinical thyroiditis”

Increased thyroidal activity on routine FDG-PET/CT after combination immune checkpoint inhibition: temporal associations with clinical thyroiditis

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Abstract

Background and objectives: FDG-PET/CT performed for purposes of response assessment complements the clinical or biochemical diagnosis of immune-related adverse events (irAEs) including thyroiditis. However, the association and temporal profile of FDG-PET/CT findings suggesting thyroiditis with the natural history of clinical and biochemical thyroid dysfunction remain unclear. This study aimed to correlate the presence and time course of FDG-PET/CT evidence of thyroiditis with the clinical diagnosis and biochemical evolution of thyroid dysfunction.

Methods: A retrospective review was performed in all patients treated with cICI for advanced melanoma between January 2016-January 2019 who underwent FDG-PET/CT at baseline and during treatment. All FDG-PET/CT scans were assessed by two independent blinded nuclear medicine physicians (NMPs) and interobserver agreement was calculated. Performance characteristics of FDG-PET/CT were defined by the receiver-operating curve (ROC) using the reference standard, thyroid function tests (TFTs). Thyroid maximum standardized uptake value (SUVmax) and its temporal changes with respect to the longitudinal biochemistry were recorded at each time point.

Results: Of 165 patients, 127 were included, with a median age of 59 years (range 18-79) and 29% were female. At a median of 3 weeks after commencing cICI, 43/127 (34%) had a diagnosis of thyroiditis established by abnormal TFTs. FDG-PET/CT was performed at baseline and at a median of 11 weeks (range 3-32) following the start of cICI. Based on blinded consensus reading, ROC showed an area under the curve of 0.87 (95% CI 0.80, 0.94) for the accuracy of FDG-PET/CT thyroiditis being confirmed by biochemical analysis. In particular, there was a high positive predictive value of 93%. Among patients with thyroiditis, those with FDG-PET/CT evidence of thyroiditis were more likely to develop overt hypothyroidism (77% versus 35%, $p<0.01$). In the evaluation of the index test, there

was an almost perfect interobserver agreement between NMPs of 93.7% (95% CI 89.4-98.0), kappa 0.83.

Conclusions: Increased metabolic activity of the thyroid on routine FDG-PET/CT performed for tumoral response of patients undergoing cICIs correlates with a biochemical diagnosis of thyroiditis despite the lag between onset of biochemical disease and the scan and is predictive of overt clinical hypothyroidism. These data suggest that an ongoing robust inflammatory response beyond the initial thyrotoxic phase may be indicative of thyroid destruction and gland failure in patients with increased thyroid FDG uptake.

Introduction

Combination immune checkpoint inhibition (cICI) with cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) inhibition has now been approved for use in patients with advanced melanoma, renal cell carcinoma and non-small cell lung cancer [2, 95] with trials continuing in other malignancies. The improved survival benefit in these previously poor prognosis malignancies with combination therapy is offset by an increase in incidence and severity of collateral immune toxicity to healthy organs, collectively termed immune related adverse events (irAEs). One of the most common irAEs after any ICI is thyroiditis[13]. Blockade of the PD-1/PD-L1 pathway is associated with an incidence of 8-25% while CTLA-4 inhibition is associated with a slighter lower incidence of 6%[4, 94]. With the combination of ipilimumab and nivolumab in the landmark Checkmate 067 trial, hyper and/or hypothyroidism occurred in 28%[9].

Unique to other forms of spontaneous thyroid autoimmunity, ICI appears to induce a rapidly destructive, inflammatory thyroiditis[61]. Subclinical hyperthyroidism, overt thyrotoxicosis and hypothyroidism may be presented separately in clinical trial safety outcomes, but most probably reflect different time points on the same disease trajectory[62].

At our center, ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) is routinely utilised in patients receiving cICI for advanced melanoma for tumor response assessment[81]. A limited number of studies indicate that new increased metabolic activity in specific organs, including the thyroid gland, on routine FDG-PET/CT imaging performed in the first three-six months of treatment may be suggestive of irAEs[82, 96]. While the finding of increased uptake of FDG in the thyroid after ICI is common[97], the temporal relationship between these findings and clinical and biochemical evidence of

thyroiditis has not been systematically evaluated. We aimed to determine the association of FDG-PET/CT findings suggestive of thyroiditis developing in response to cICI as compared to the reference standard of biochemical assessment of TFTs. We further sought to evaluate interobserver agreement for FDG-PET/CT as a novel modality for the detection of thyroiditis and derive semiquantitative cut-offs values for thyroiditis.

Methods

This retrospective study was approved by the institutional Ethics Committee (approval number: 17/231R). A pharmacy dispensing report was used to identify all patients who received at least one dose of cICI for metastatic melanoma between January 2016- January 2019 at a quaternary melanoma center in Melbourne, Australia. Patients were eligible for inclusion if they had available FDG-PET/CT imaging at baseline and within 6 months of commencing cICI. Patients who had received prior first-line therapy with single agent anti-PD-1, anti-CTLA-1 or BRAF/MEK inhibition were included. Patients with a history of thyroiditis related to a prior line of immunotherapy were excluded. A chart review was undertaken to identify patients diagnosed with thyroiditis and the corresponding clinical presentation, thyroid function test (TFT) results and thyroid autoantibodies were recorded.

Test methods

Reference standard

In keeping with the American Society of Clinical Oncology (ASCO) guidelines, patients receiving cICI had TFTs at baseline, at every cycle for the first 4 cycles, every 4-6 weeks thereafter and earlier in the case of clinical suspicion. TFTs included thyroid stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) and were performed on the Abbot Architect chemiluminescent immunoassay (Abbott Diagnostics, Abbot Park, IL). In-house

laboratory pre-specified population reference ranges were utilised for TSH (0.35-4.94mU/L), T4 (9-19 pmol/L) and T3 (2.6-5.7pmol/L). Biochemical thyroiditis was defined as 1) subclinical thyrotoxicosis (low TSH, normal T3 and T4 in the absence of suspected hypophysitis), 2) overt thyrotoxicosis (low TSH, elevated T3 and/or T4), 3) subclinical hypothyroidism (high TSH, normal T3 and T4) or 4) overt hypothyroidism (high TSH, low T3 or T4). Time to thyroiditis, duration of the thyrotoxic phase and percentage developing permanent hypothyroidism were retrospectively assessed. When measured, levels of anti-thyroperoxidase antibodies (TPOAb) and anti-thyroglobulin antibodies (TgAbs) were performed on an electrochemiluminescent immunoassay (Elecsys Anti-TPO kit and Elecsys Anti-Tg kit, respectively; Roche diagnostics, Mannheim Germany.)

Index test

All studies were performed on an integrated PET/CT scanner including Biograph 16 (Siemens Medical Solutions, Erlangen, Germany), GE Discovery 690 and GE Discovery 710 (GE Healthcare, Milwaukee, WI), with routine cross-calibration three-monthly. FDG-PET/CT was performed as per European Association of Nuclear Medicine Research Ltd. (EARL) initiative[89]. Although there are no established criteria for the diagnosis of thyroiditis based on FDG-PET/CT, a normal thyroid demonstrates no increased metabolic activity above the background and should not be seen as a discrete organ on PET images. Therefore, a visible thyroid on FDG-PET/CT images which was new compared to the pre-treatment study was considered suggestive of thyroiditis. Two experienced nuclear medicine physicians (NMPs) blinded to clinical data and the reference standard results assessed all available FDG-PET/CT scans independently. Majority agreement was used to define findings suggestive of thyroiditis. If only one NMP noted an increase in FDG uptake, the assessment was considered negative for thyroiditis. The interobserver agreement for FDG-PET/CT

interpretation of thyroiditis was evaluated. In addition, a semiquantitative analysis on PET images was performed by a 1 cm^3 spherical volume of interest over the thyroid gland, as identified on the CT portion of PET/CT, using MIM software (MIM 6.7.11; MIM Software, Cleveland, OH). The maximum standardised uptake value (SUVmax) in the thyroid gland was measured at baseline, after initiation of cICI and until resolution and percentage increase in SUVmax from baseline was compared.

Analysis

The standard of truth for thyroiditis was determined by TFTs. Clinical variables in patients with and without thyroiditis were compared using Fisher's exact, χ^2 and Wilcoxon rank sum tests. Time to thyroiditis and duration of the thyrotoxic phase were described as continuous variables with median and interquartile ranges (IQRs). The duration of the thyrotoxic phase was defined as the time in weeks from first TFT evidence of subclinical or overt thyrotoxicosis to normalisation of TFTs or a TSH above the upper limit of normal (which ever occurred first). Patients diagnosed with thyroiditis who had indeterminate follow up results were excluded from the analysis of duration of the thyrotoxic phase.

The result of the index test was listed as a binary positive or negative. For the purpose of the analysis, equivocal results (one user only) were classified as negative. The diagnostic accuracy of FDG-PET/CT with respect to the reference standard was represented as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals. The AUC for overall performance of FDG-PET/CT was calculated as the mean of the estimated sensitivity and specificity by reported operating characteristics (ROC). Interobserver agreement for the diagnosis of thyroiditis by FDG-PET was assessed by the Cohen's kappa statistic. The optimal cut-offs for thyroid SUVmax and its

temporal changes suggestive of thyroiditis were selected based on a predictive model for optimal sensitivity and specificity. All statistical analyses were performed using STATA version 16.1 (STATA LP College Station, Tx, USA).

Results

Participants

Of 162 patients treated with combination ICIs from January 2016 to January 2019, 127 patients met the inclusion criteria for the study. 28 patients did not have accessible post-treatment FDG-PET imaging and 7 patients were excluded due to a prior history of thyroiditis. The median age was 59 years (range 18-79) and 29% were female. Overall, 43/127 (34%) had a diagnosis of thyroiditis established with TFTs (Figure 1.). There was no difference in patient demographics between those with and without thyroiditis (Table 1.). Patients with thyroiditis were less likely to have received prior treatment with pembrolizumab (9% versus 31%, P=0.007), while there was no difference in prior exposure to nivolumab or ipilimumab monotherapy. A higher proportion of patients with thyroiditis had previously received BRAF/MEK inhibition (58% versus 31%, p= 0.003)

Figure 1. Consort diagram

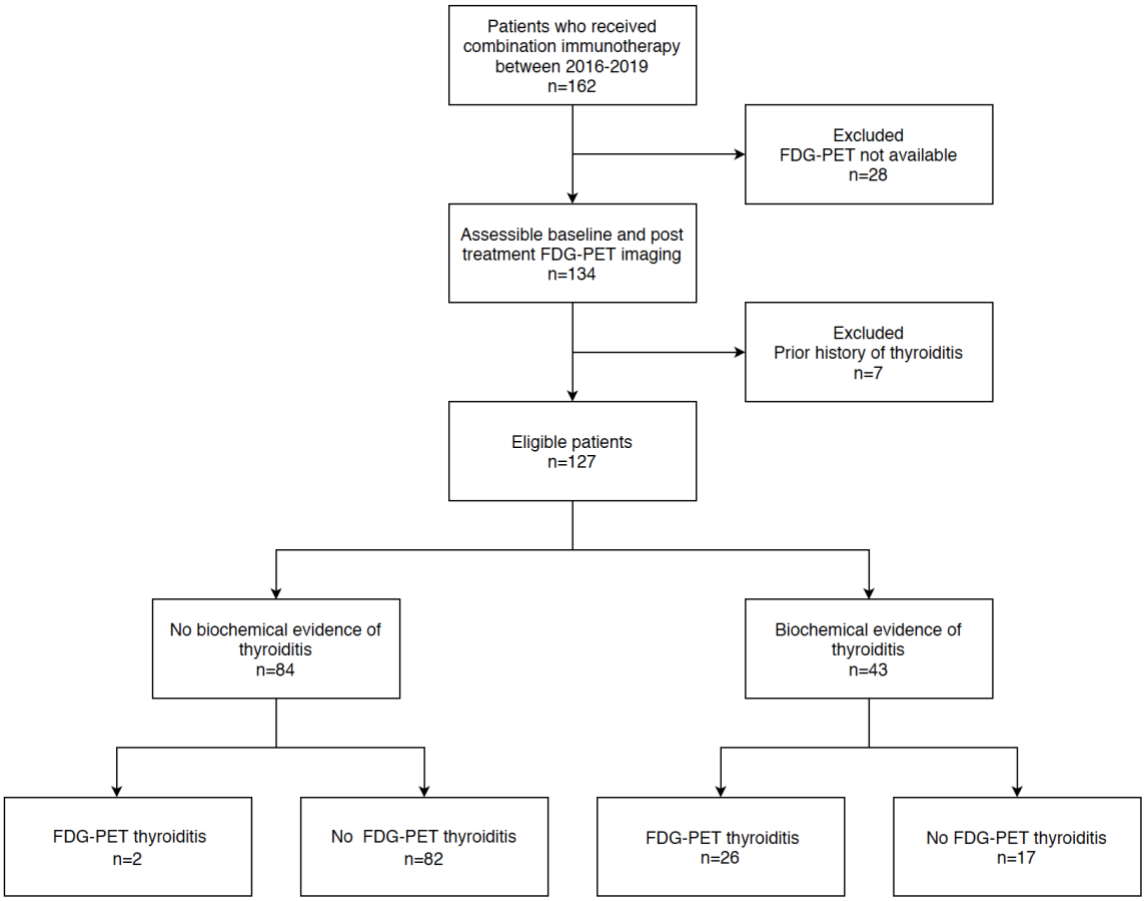


Table 1. Clinical Characteristics

	Thyroiditis (n= 43)	**No Thyroiditis** (n= 84)	**P value**
No. (%) Male	29 (67.4%)	61 72.6%)	0.54
No. (%) Female	14 (32.6%)	23 (27.4%)	
Age, median (IQR), years	58 (50-67)	61 (47-69)	0.99
BMI, median (IWR), kg/m^2	28 (25-34)	27 (24-30)	0.055
to single agent ICI or targeted immunotherapy No. (%) prior exposure			
Ipilimumab	1 (2.3%)	5 (6.0%)	0.36
Nivolumab	0 (2.0%)	4 (4.8%)	0.15
Pembrolizumab	4 (9.3%)	26 (31.0%)	0.007
BRAF/MEK Inhibition	25 (58.1%)	26 (31.0%)	0.003
No. (%)Prior Autoimmunity (where specified)			
≥1 autoimmune condition	3 (7.0%)	10 (11.9%)	0.39
Rheumatoid Arthritis	0 (0.0%)	2 (2.4%)	0.31
Inflammatory Bowel Disease	1 (2.3%)	1 (1.2%)	0.63
Psoriasis	0 (0.0%)	2 (2.4%)	0.31
Vitiligo	1 (2.3%)	0 (0.0%)	
Alopecia	0 (0.0%)	1 (1.2%)	
Sjogren's Syndrome	0 (0.0%)	1 (1.2%)	
Graves' Disease	0 (0.0%)	0 (0.0%)	
Hashimoto's Thyroiditis	0 (0.0%)	2 (2.4%)	
Coeliac Disease	0 (0.0%)	1 (1.2%)	
Type 1 Diabetes	0 (0.0%)	1 (1.2%)	
Multiple Sclerosis	0 (0.0%)	0 (0.0%)	
Behcet's Disease	0 (0.0%)	1 (1.2%)	
Immune Glomerulonephritis	1 (2.3%)	0 (0.0%)	
Aortitis	0 (0.0%)	1 (1.2%)	0.47
Immune related adverse events			
No. (%) with ≥1 irAE (other than thyroiditis)	40 (90.1%)	74 (88.1%)	0.26
No. (%) with at least one grade 3/4 irAE	23 (52.3%)	35 (41.7%)	0.27
No. (%) by organ system			
Hypophysitis	9 (20.9%)	18 (21.4%)	1.0
Dermatological	22 (51.2%)	39 (46.4%)	0.71
Hepatitis	14 (32.6%)	26 (31.0%)	0.84
Enteritis/Colitis	14 (32.6%)	28 (33.3%)	1.0
Rheumatic	7 (16.3%)	8 (9.5%)	0.38
Pneumonitis	5 (11.6%)	9 (10.7%)	1.0
Nephritis	1 (2.3%)	3 (3.6%)	1.0
Myocarditis	0 (0.0%)	1 (1.2%)	1.0
CNS including eye and ear	2 (4.7%)	3 (3.6%)	1.0
Other (Lymphadenitis, Haemolytic Anaemia, Panniculitis-like T-cell Lymphoma)	1 (2.3%)	3 (3.6%)	1.0

Established thyroiditis

Thyroiditis was diagnosed at a median of 3 weeks from commencement of cICI (range 1-15). 37/43 patients had a documented thyrotoxic phase which was overt in 34 and subclinical in 3, with a median duration of 4.5 weeks. The thyrotoxic phase was transient in all cases. 6 patients presented with hypothyroidism as the first abnormal TFT, and 19/37 patients with a transient thyrotoxic phase progressed to a hypothyroid phase. Overall, long term thyroxine was commenced in 23/43 patients. Once commenced, thyroxine withdrawal was not attempted by the treating clinicians. The majority of patients (38/43) were managed in the outpatient setting with mild symptoms. 5 patients required hospital admission during the thyrotoxic phase of which 4 were primarily receiving treatment for another contemporaneous irAE. Two patients complained of mild transient neck pain which did not require treatment with glucocorticoids. TPO and TG ab were measured in 17/43 patients and were elevated in 6 (35%) and 11(65%) respectively. TSHrAb was measured in 13 people. Only one individual had a high titre of TSHrAb >40 IU/L (reference range <1.8IU/L). Thyroid hormone levels in this individual followed a pattern of minimally symptomatic transient thyrotoxicosis followed by permanent hypothyroidism. In this case, the presence of TSHrAb did not herald prolonged thyroid hormone overproduction.

Index test results

FDG/PET-CT was performed in all patients at a median of 11 weeks from commencement of cICI (range 3-32). FDG/PET-CT was declared positive by two NMPs in 26/43 (60%) patients with thyroiditis but only 2/84 (2%) patients not diagnosed with thyroiditis (Figure 1. and Table 2.), resulting in an AUC of 0.87 [95% CI 0.8, 0.94]. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 61% [95%

confidence interval (CI) 44-75], 98% [95% CI 92-100%], 93% [95% CI 77-91] and 83% [95% CI 74-90] respectively.

Table 2. Cross tabulation of the index test results by the results of the reference standard

	Reference test +	Reference test -	Total
Index Test +	26	2	28
Index Test -	17	82	99
Total	43	84	127

Typical evolution of the thyroid SUVmax in a patient with thyroiditis is demonstrated in Figure 2. In 22/28 patients with PET-thyroiditis and available further follow-up FDG PET/CT, resolution of SUVmax to baseline was noted in all cases by a median of 28.6 weeks (range 17.9-158) from the start of cICIs.

Figure 2. Typical evolution of thyroid FDG uptake in a patient with cICI related thyroiditis

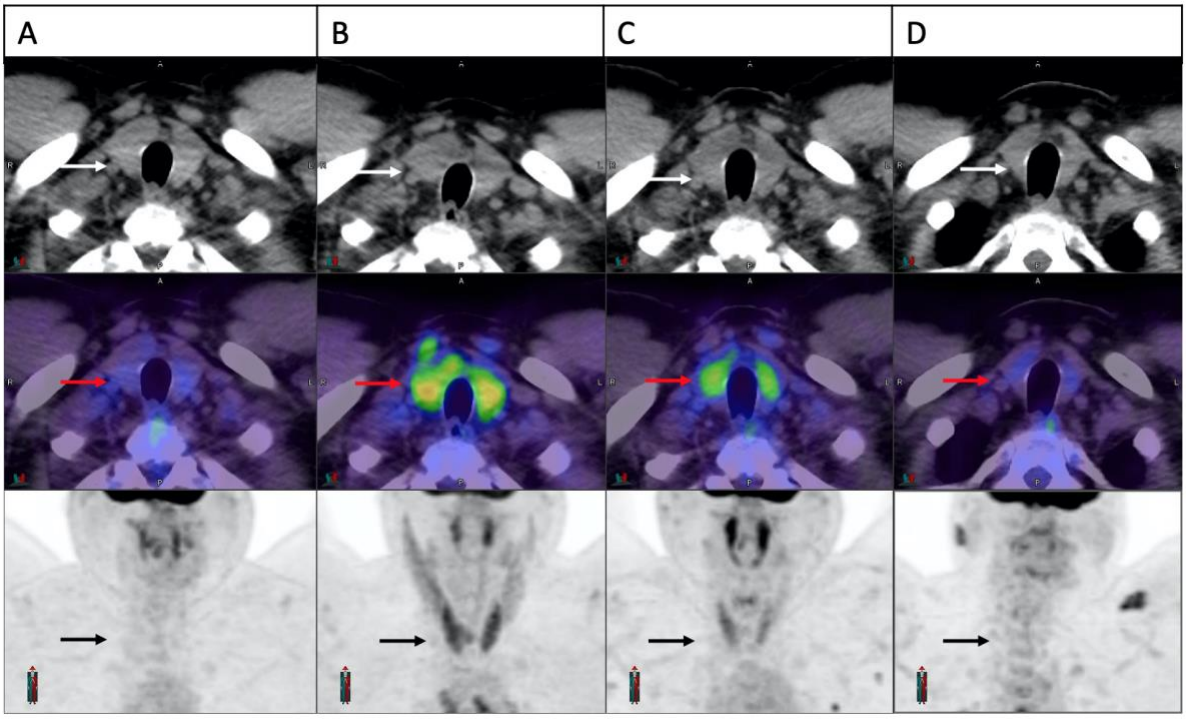


Figure legend: Trans-axial and maximum intensity projection images of the thyroid gland demonstrated in sequence on CT scan (white arrows), fusion image (red arrows) and PET scan (black arrows). Normal thyroid appearance is demonstrated at baseline (panel A). A significant increase in uptake is noted on first on-treatment scan at three weeks (panel B). Panel C and D demonstrate partial and complete resolution at 6 weeks and 24 weeks, respectively.

Interobserver agreement

Both users agreed on 93.7% of index test results [95% CI 89.4-98.0] (Table 3). The Cohen's Kappa score was 0.83, indicating almost perfect interobserver agreement between both users according to scale provided by Landis and colleagues[98].

Table 3. Cohen's Kappa score

	User 1: Index test +	User 2: Index test -	Total
User 2: Index test +	28	5	33
User 2: Index test -	3	91	94
Total	31	96	127
Agreement: 93.7% [95% CI 89.4-98.0], Cohen's k: 0.3 (almost perfect agreement)			

Semiquantitative analysis

The median baseline thyroid SUVmax in the cohort was 1.8 (IQR 1.5-2.1). When both users agreed on the qualitative diagnosis of thyroiditis, the median SUVmax at first follow-up scan was 5.15 (IQR 3.95-6) with a corresponding absolute and % increase of 2.7 (IQR 1.9-3.8) and 127% (IQR 89-190%) respectively. In patients with equivocal results (one user only), the median SUVmax at first follow up was 2.8 (IQR 2.45-2.95), with a corresponding absolute and % increase of 1.1 (IQR 0.35-1.35) and 61% (IQR 18-88%) respectively. When both users agreed on a negative result, the median SUVmax at first follow up was 1.6 (IQR 1.4-1.9) with a median change of 0% (IQR -16-10%) (Figure 3.). Equivocal results lead to a small number of discordant results in the Kappa agreement score. These reflect nuanced reporting in the lower range of abnormality, rather than the abnormality being detected by one user and not the other.

Figure 3. Box plot SUVmax and %change SUV max

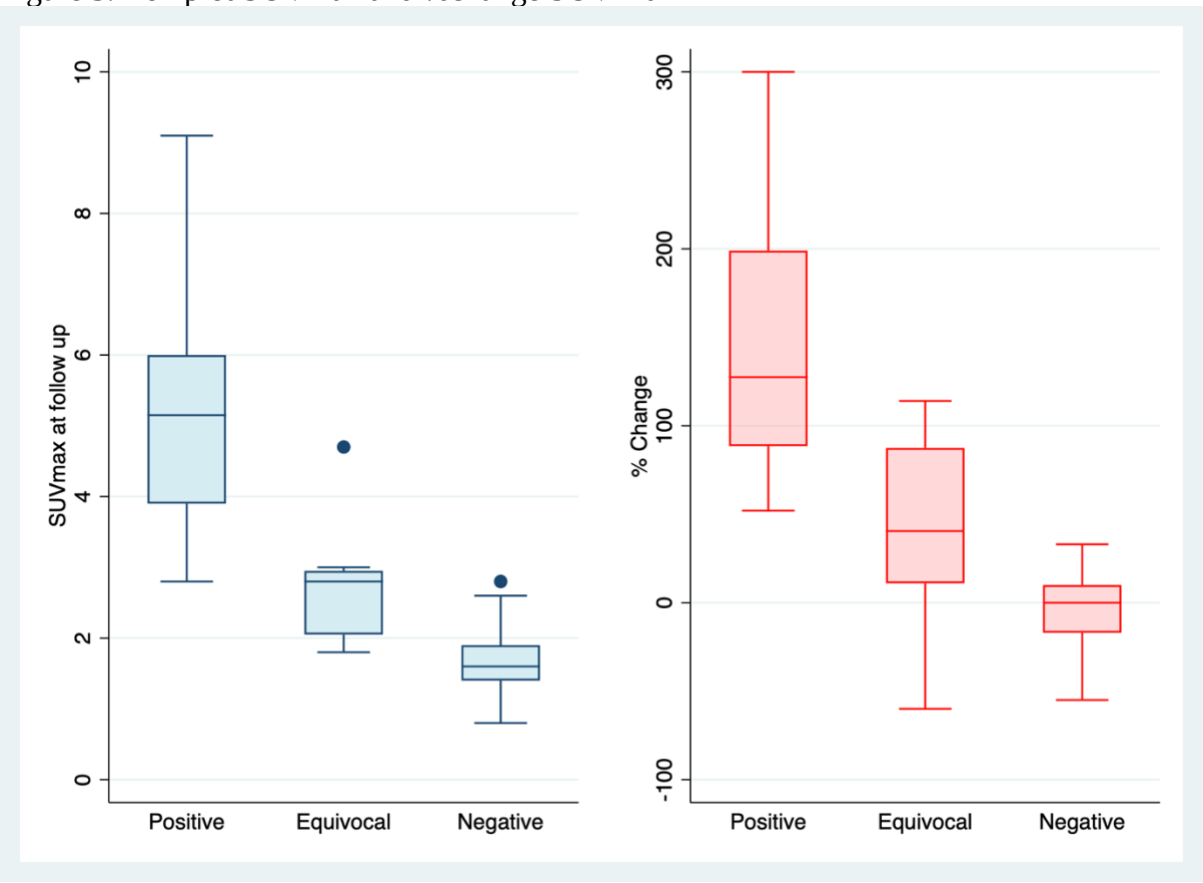
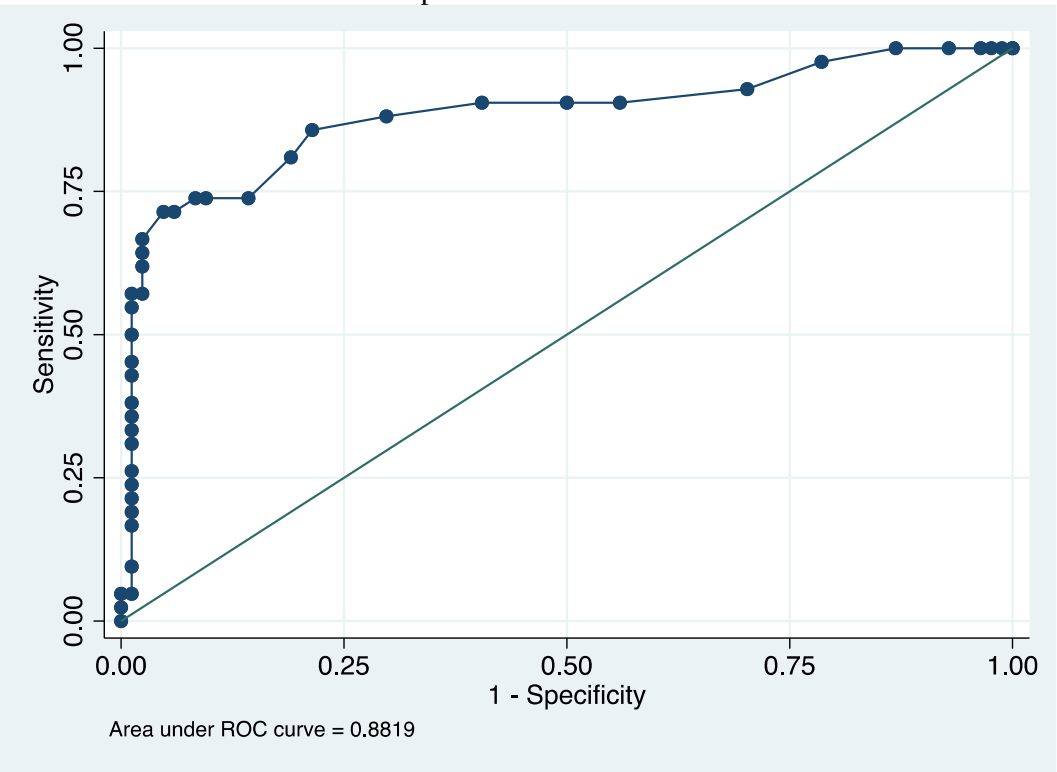


Figure Legend:
Absolute SUVmax at the first on-treatment FDG-PET/CT, and % change in SUVmax with respect to baseline in patients where FDG-PET/CT detected thyroiditis was documented by both NMPs (positive), by one NMP (equivocal) and by neither NMP (negative). Equivocal results were deemed to reflect nuanced reporting in the lower range of abnormality and were classified as negative in the analysis.

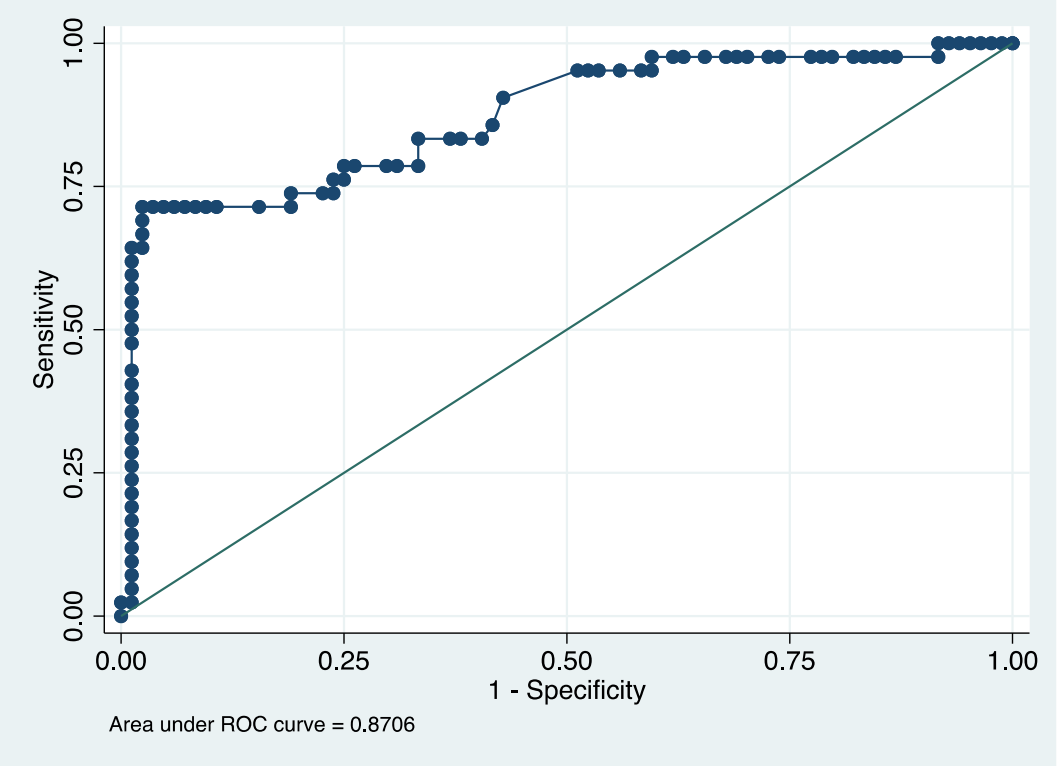
Using a predictive model, the SUVmax cut off of 2.8 infers a sensitivity of 71%, with a specificity of 95% and correctly classifies thyroiditis in 87% of patients. The SUVmax (% change) cut off of 52% infers a sensitivity of 71%, specificity of 98% and correctly classifies thyroiditis in 89% of patients. These cut offs increase the overall test sensitivity from 61% with a small decrease in specificity for SUVmax from 98% but no change to specificity using %change. Receiver operator curves for SUVmax and %change are presented in Figure 4.

Figure 4. Receiver Operator Curves

A. SUVmax at first follow up scan



B. SUVmax% Change



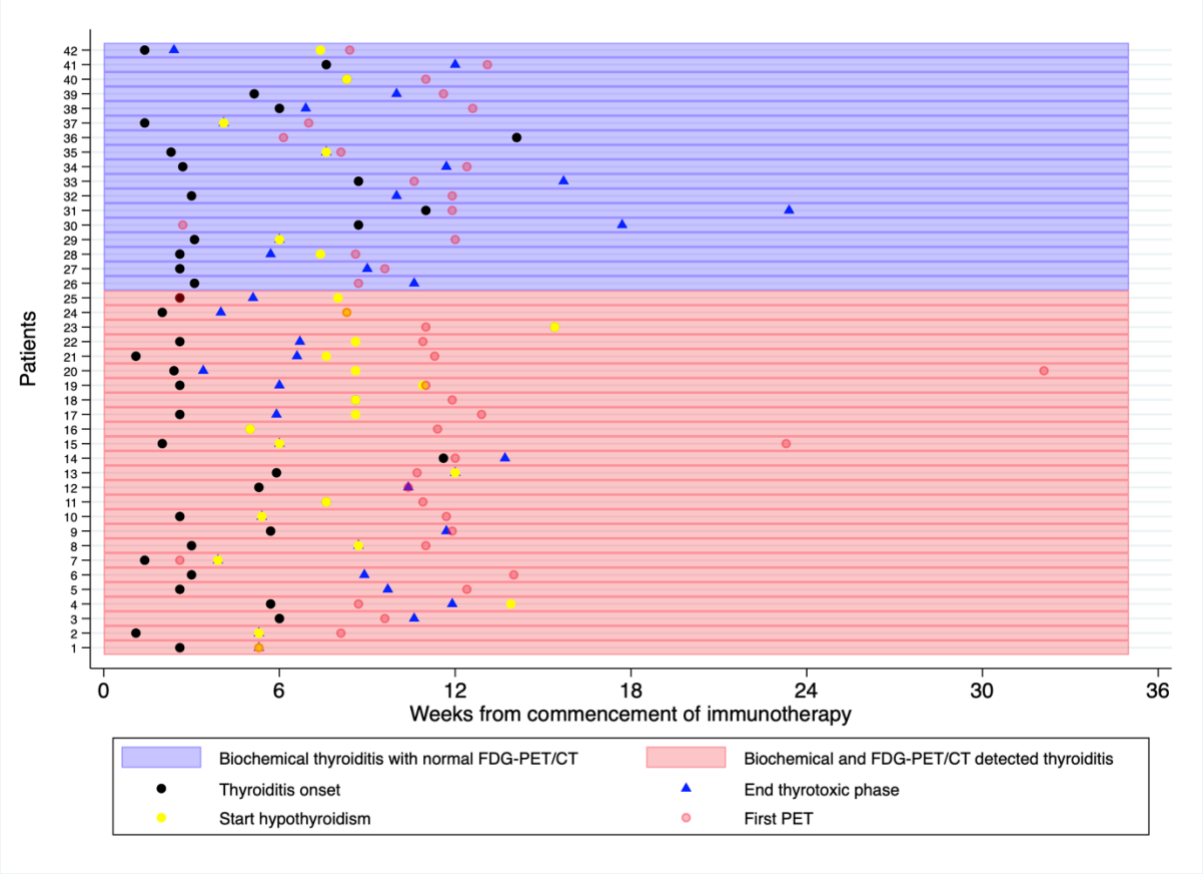
Clinical correlation

The timing of FDG-PET/CT with respect to the biochemical diagnosis is illustrated in Table 4. and Figure 5. In almost all cases, the biochemical diagnosis preceded the first on treatment FDG-PET/CT. There was no difference in the time interval between biochemical thyroiditis and the first FDG-PET/CT in patients with and without PET detected thyroiditis.

Table 4. Timing of imaging with respect to the biochemical diagnosis

	Time to biochemical diagnosis (weeks)	Time to FDG-PET/CT (weeks)	Time interval (weeks)
Biochemistry + PET + N=26	Median 2.8 (range 1.1-97)	Median 11 (range 2.6-32.1)	Median 6.35 (range 0-86.7)
Biochemistry + PET – N=17	Median 3.1 (range 1.4-14.1)	Median 10.6 (range 2.7-13.1)	Median 6 (range 0.9-9.7)
P value	0.34	0.57	0.62

Figure 5. The temporal relationship between clinical thyroiditis and the first FDG-PET/CT



Overall, patients with FDG-PET-detected thyroiditis were more likely to develop overt hypothyroidism (71% versus 6%, $p<0.001$). In the cohort of patients with established biochemical thyroiditis, this relationship was maintained (35% versus 77%, $p<0.01$).

One patient had an abnormal PET at baseline (2 months prior to cICI), which resolved on the first follow up study (Figure 6.)

Figure 6. Increased thyroid FDG uptake at baseline in a patient who developed cICI related thyroiditis

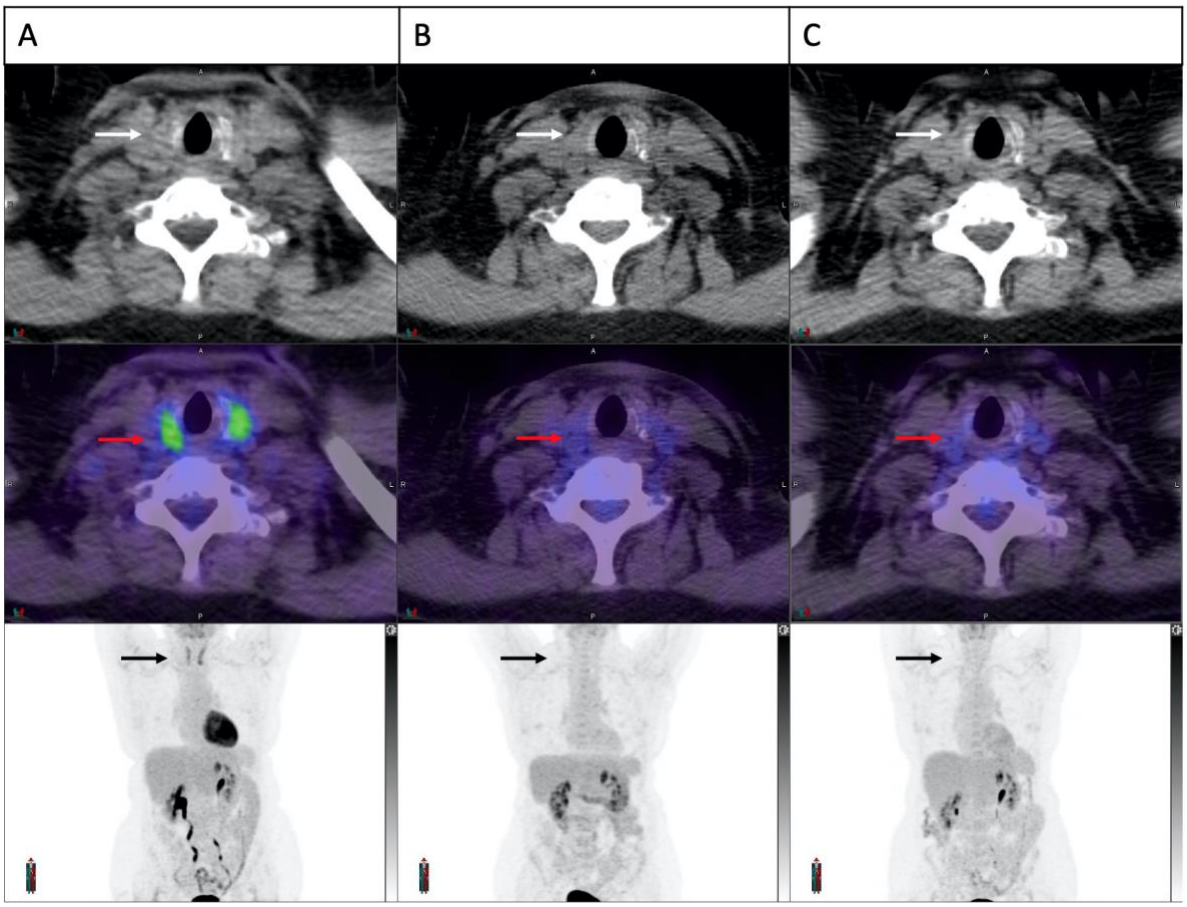


Figure legend: Trans-axial and maximum intensity projection images of the thyroid gland demonstrated in sequence on CT scan (white arrows), fusion image (red arrows) and PET scan (black arrows). A clear increase in thyroid uptake is demonstrated at baseline (panel A), which had resolved completely on both on-treatment scans at 6 and 14 weeks (Panel B and C). The patient was euthyroid at baseline and developed biochemical thyroiditis 10 days after commencement of cICI.

This patient had normal thyroid function at the time of the baseline PET and developed severe thyrotoxicosis (T4 138pmol/L, TSH <0.01mU/L) 10 days after commencing cICI, 2 months after the abnormal baseline PET and 1.5 months before the normal follow up PET. Autoantibodies were not measured. No prior anti-PD-1 or anti-CTLA-4 therapy had been administered in this case, indicating a probable existing subacute autoimmune thyroiditis. There were two patients without thyroiditis who had an abnormal FDG-PET/CT result. One patient was diagnosed with hypophysitis and secondary hypothyroidism. While there was no thyrotoxic phase, subsequent primary hypothyroidism could not be excluded. The second patient had normal TFTs two weeks before and one month after the abnormal PET and remained euthyroid.

Thyroid myopathy

In the 28 patients with FDG-PET/CT detected thyroiditis, there were two patients with overt evidence of PET myopathy. In both cases, transient diffuse skeletal muscle uptake occurred concurrently with increased thyroid uptake and biochemical thyroiditis, suggesting a potential diagnosis of thyroid myopathy (Figure 7). One patient remained asymptomatic while the other experienced significant proximal weakness for the duration of the thyrotoxic phase. This patient developed biochemical thyrotoxicosis 9 days after commencement of cICI, while receiving high-dose glucocorticoid treatment for cerebral metastases. Autoantibodies to TPO and TG were elevated. Over the following 3 weeks, progressive generalised muscle weakness was reported despite a decreasing dose schedule of glucocorticoids. Random cortisol and serum creatine kinase levels were normal. FDG-PET/CT performed two months after treatment commencement demonstrated interval development of thyroid and diffuse skeletal muscle uptake. The weakness resolved after transition to the hypothyroid phase.

Figure 7. Diffuse skeletal muscle uptake of FDG in a patient with thyroiditis

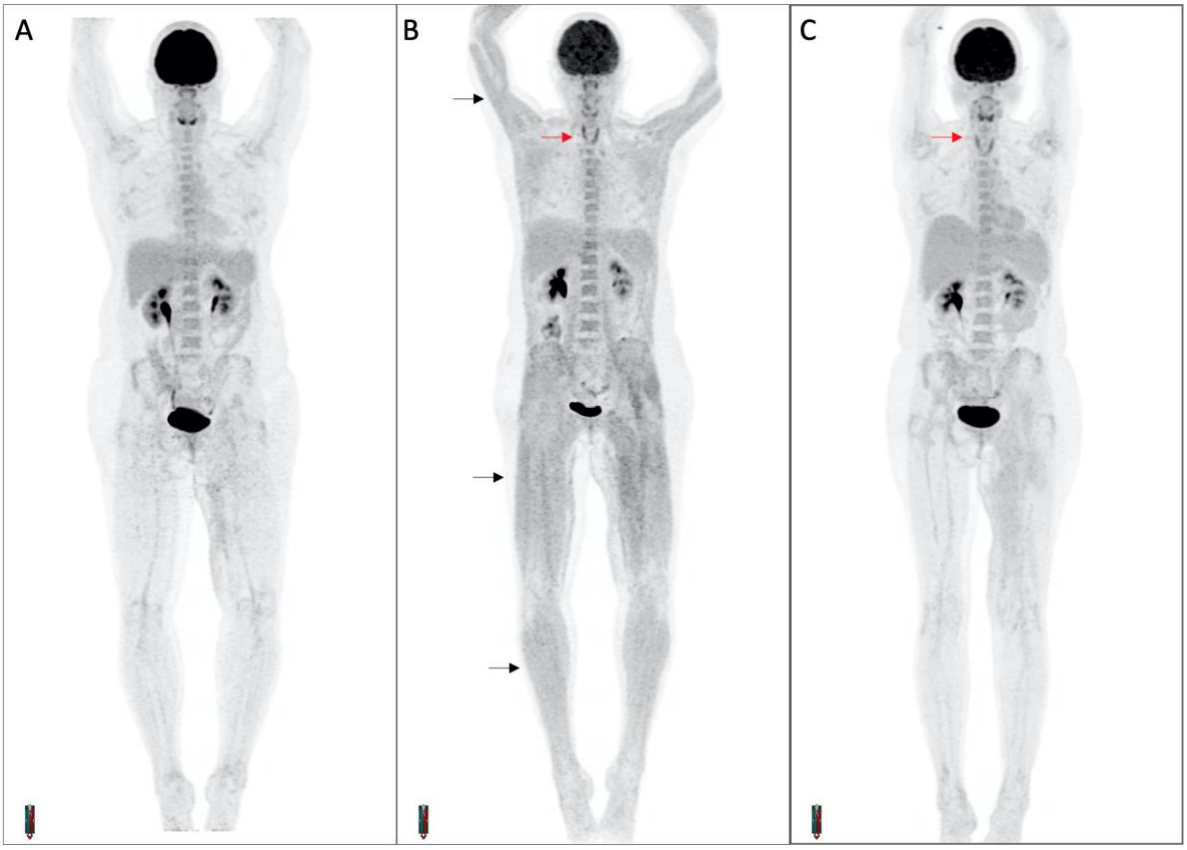


Figure Legend: Maximum intensity projection PET images at baseline (A) and on-treatment (B and C) demonstrate interval development of thyroiditis (red arrows) and diffuse skeletal muscle uptake (black arrows). The patient complained of progressive upper and lower limb weakness during the thyrotoxic phase, with a normal CK.

Discussion

cICI related thyroiditis was common at our center, with a prevalence similar to the combination arm of the CheckMate 067 study (34% compared to 28%). FDG-PET/CT had a high specificity and positive predictive value for thyroiditis with respect to the reference standard biochemical test, although the temporal profile of biochemical and scan findings are out of sync. With the initial thyrotoxic phase generally having been detected prior to PET evaluation and being largely resolved by the time FDG PET/CT demonstrated evidence of ongoing increasing FDG uptake, our findings suggest that the thyroiditis continues beyond the phase of initial inflammatory hormone release with an ongoing process of thyroid destruction. Biochemical diagnosis of thyroiditis occurred early, at a median of 3 weeks from

commencement of cICI. The lower sensitivity of FDG-PET/CT may relate to the timing of the imaging with respect to onset of the thyroiditis. With the median time of the first scan 11 weeks, most patients had already developed biochemical abnormality and, in a subgroup of patients, the acute inflammatory phase (as represented by increased FDG uptake in the gland) may have resolved by the time of the first scan. The comparison of time interval between patients with biochemical thyroiditis with and without PET changes was, however, not different. Importantly, the patients who had FDG-PET/CT evidence of thyroiditis had a higher likelihood of developing overt hypothyroidism requiring ongoing thyroid replacement. Hence, we have stipulated that presence of increased FDG activity may indicate a more robust autoimmune response and destruction of thyroid gland by the adoptive immune system. Whether thyroid hormone replacement could be withdrawn when FDG uptake in the gland had resolved was not tested in this population with clinicians continuing treatment once initiated.

Pathophysiology of thyroiditis

Currently, there is very little data as to the pathophysiology of ICI related thyroiditis. In our cohort, TPOAb and TgAb were elevated in 35% and 65% respectively, compared to the expected 95% and 80% reported in spontaneous autoimmune thyroid disease[63]. New onset Graves' disease after ICI is rare but has been described in case reports, and guidelines do recommend measuring TSHrAb in any case of severe or symptomatic thyrotoxicosis to guide initiation of antithyroid drugs[31, 99, 100]. The one patient with a high titre of TSHrAb in our cohort experienced a brief thyrotoxic phase but this was followed by permanent hypothyroidism. The heterogeneity in the presence of thyroid autoantibodies suggests that autoantibodies are not the main mechanism behind thyroid-specific autoimmunity after ICI. Rather, a pathogenic T cell response may be accompanied by an antibody response. As a

glucose analogue radiotracer, FDG uptake in thyroiditis may reflect the increase glycolytic activity of activated CD8+ t cells infiltrating the gland[83]. Cytological and molecular analyses of thyroid tissue would be of benefit in understanding the underlying mechanisms however thyroid biopsy in otherwise recovering patients is difficult to justify[13].

The absence of evidence of thyroid hormone overproduction in ICI-related thyroiditis prompts the recommendation that if anti-thyroid drugs are commenced due to an elevated TSHrAb, TFTs should be monitored within 2 weekly intervals with a low threshold to cease treatment as the spontaneous thyrotoxic phase resolves.

FDG-PET/CT in ICI related thyroiditis

A limited number of studies have reviewed the role of FDG-PET/CT in ICI related thyroiditis. At our centre, we have previously demonstrated the accuracy of FDG-PET/CT in detecting both tumoral and systemic immune response. In our prior study, increased uptake in the thyroid following treatment was found in 6 patients, all of whom had biochemical correlation[82]. Yamauchi and colleagues reviewed results from baseline FDG-PET/CT imaging performed prior to ICI administration in 111 patients. A significant correlation between increased thyroid uptake before treatment and development of thyroiditis after treatment was observed (46.7% versus 4.2% $p<0.001$)[96]. In our current study, all patients had TFTs prior to cICI and we excluded patients with a prior history of thyroid dysfunction. However, we did find evidence of pre-treatment FDG-PET/CT detected thyroiditis in one patient who went on to develop new thyrotoxicosis soon after the first dose of cICI. In a small study of 18 lung cancer patients treated with nivolumab, baseline imaging was not associated with thyroiditis, however increased uptake in the thyroid 10-16 weeks after treatment commencement was predictive of hypothyroidism before the TSH became

elevated[97]. In that study, only 6 patients had thyroiditis. The finding that the presence of thyroid metabolic activity on FDG-PET/CT is predictive of overt hypothyroidism was replicated in our study with a larger number of patients.

While the discrepancy between onset of biochemical thyroiditis and timing of FDG-PET/CT findings suggestive of this diagnosis is a limitation of the clinical utility of this finding, the incidental observation of increased thyroid uptake should initiate careful monitoring of thyroid function if not already known to be abnormal. Whether thyroid hormone replacement can be safely withdrawn after resolution of active thyroid uptake of FDG on PET remains unknown.

Conclusions

The primary role of FDG-PET/CT is monitoring of tumoral response and not for the evaluation of thyroiditis. However, with an increasing incidence of thyroid abnormalities detected on routine FDG-PET/CT imaging it is of great importance to understand the implications of these findings. Although routine FDG-PET/CT is most commonly performed after onset of the thyrotoxic phase, our study demonstrates that FDG-PET/CT has a high specificity and positive predictive value for ICI related thyroiditis and is predictive of progression to permanent hypothyroidism. We demonstrate high interobserver agreement for the detection of thyroiditis with FDG-PET and describe semiquantitative cut-off values using SUVmax and %change with respect to baseline. To our knowledge, this is the first study to systematically evaluate the temporal profile of FDG-PET/CT findings of thyroiditis in relation to the biochemical and clinical course of thyroid dysfunction after cICI.

4.3 Conclusion

Thyroiditis after cICI was common at our centre, with a prevalence similar to the combination arm of the CheckMate 067 study (34% compared to 28%). This is an irAE that presents early after commencement of treatment, most often with a transient thyrotoxic phase. Our findings confirm that in the majority of patients, antithyroid drugs are not of use as this does not appear to be a disorder associated with thyroid hormone over production. Even in the setting of a very high TSHrAb titre, the thyrotoxic phase very quickly progresses to hypothyroidism. Having said that, Graves' disease remains relatively prevalent in the general community and should be considered if the thyrotoxic phase persists past 2-4 weeks.

We found that FDG-PET/CT had a high specificity and positive predictive value for thyroiditis with respect to the reference standard test. The lower sensitivity may relate to the discrepancy in timing between median onset of thyroiditis (3 weeks) and the median timing of first follow up scan (11 weeks). While the discrepancy between onset of thyroiditis and timing of FDG-PET/CT is a limitation of our diagnostic accuracy study, these findings are generalisable to other major cancer centers, where FDG-PET/CT would be performed with similar timing. After determining that patients who had FDG-PET/CT evidence of thyroiditis had a higher likelihood of developing overt hypothyroidism requiring ongoing thyroid hormone replacement, we have stipulated that presence of increased FDG activity may indicate a more robust autoimmune response and destruction of thyroid gland by the adoptive immune system.

Overall, our findings support existing guidelines which suggest routine monitoring of thyroid function tests at 4-6 week intervals after ICI. In the setting of symptomatic thyrotoxicosis, management with beta blockers is considered first line therapy[31]. Cautious introduction of

antithyroid drugs may be instituted for severe thyrotoxicosis, however repeat thyroid function tests at short intervals (ie fortnightly) are required with prompt withdrawal of treatment on resolution of the thyrotoxic phase. Attempts to withdraw thyroxine at a later date were not made in our cohort but could be considered in patients followed up in the endocrine clinic to assess for thyroid recovery.

Chapter 5. Conclusions and future directions

5.1 Summary of key findings

The research conducted in this thesis contributes to the growing number of evolving recommendations regarding the recognition and management of diabetes, hypophysitis and thyroiditis in the setting of combination ICI.

Diabetes and pancreatitis

First, I have described the phenotype of CPI-DM as an accelerated, fulminant islet autoimmunity resulting in a brittle, insulin dependent and ketosis-prone form of chronic diabetes. Primary care physicians, emergency doctors, oncologists and endocrinologists need to be aware of this condition in order to implement prompt management with insulin to avoid the development of hyperglycaemic emergencies and their associated significant need for intensive care management and avoidance of a potentially fatal outcome.

I then describe the challenges in recognising CPI-DM, particularly if a patient presents atypically, with the sub-acute onset of symptoms or with confounding factors such as the use of high-dose glucocorticoids or an SGLT-2 inhibitor, which are both known to induce ketosis. I also compared the results of pancreatic imaging in our cohort to rare case-reports, to build an understanding of the significance of pancreatic imaging changes in relation to endocrine and exocrine pancreatic function. The ability to recognise CPI-DM as distinct from other causes of hyperglycaemia in cancer patients will be critical when considering future therapeutic strategies for the reversal of islet destruction after ICI.

Hypophysitis

Hypophysitis has been considered an irAE largely confined to patients treated with CTLA-4 inhibitors. However, in a cohort of patients treated with combination CTLA-4 and PD-1 inhibition, we found a very high incidence of both clinically recognised hypophysitis and isolated imaging features consistent with the presence of pituitary inflammation. Despite a high vigilance for monitoring of cortisol and thyroid function levels at our centre, I still identified a significant lack of testing of a full anterior pituitary hormone panel, particularly of the gonadal axis. There was also a lack of longitudinal monitoring of pituitary hormones after diagnosis, or any attempt to withdraw hormone replacement to evaluate for recovery of pituitary function. These findings have been conveyed to the oncology team with recommendations for the evaluation anterior pituitary function. Our findings relating to pituitary imaging features on CT, MRI and FDG-PET/CT provided a novel insight into the role of routine cancer imaging in the detection of pituitary inflammation. We suggest imaging criteria for the diagnosis of ICI related hypophysitis. This approach has not previously been evaluated in the published literature to date.

Thyroiditis

A detailed description of the natural history of thyroiditis after cICI contributes to a larger body of work in the literature describing this form of thyroid dysfunction as an inflammatory thyroiditis which occurs early after treatment, often with a thyrotoxic phase and ultimately leading to hypothyroidism in the majority of patients. In the absence of standardised imaging criteria, I presented a diagnostic accuracy study evaluating the role of FDG-PET/CT, performed for the indication of tumour re-staging, in the diagnosis of thyroiditis. Our findings demonstrated that FDG-PET/CT has a high specificity for thyroiditis and is predictive of overt hypothyroidism. FDG-PET/CT is increasingly used in the staging of cancers and for the evaluation of the response to cancer treatments, therefor understanding the implication of

incidental changes in thyroid uptake and their temporal associations with clinical and biochemical thyroiditis would appear to be an important step in evaluating a patients' chances of developing clinical important thyroid dysfunction associated with ICI therapy.

5.2 Conclusions

In this thesis I have explored important clinical, immunological and radiological features of ICI related diabetes, hypophysitis and thyroiditis. These studies were performed at a large melanoma referral centre, The Peter MacCallum Cancer Centre in Melbourne, Australia. Data obtained regarding the incidence and natural history of these endocrine toxicities have great impact on patient care, education and follow up in this cohort of people living with complex health problems already. In Melbourne, many patients travel from regional areas to receive specialist oncological care, however, they will often present locally for primary care and for after-hours emergencies. Patient education, early recognition and prompt treatment of the endocrinopathies associated with ICI use may result in fewer hospital admissions and reduced morbidity in patients undergoing cancer treatments.

Identifying a set of diagnostic criteria for the radiological diagnosis of endocrine irAEs should be of great value in optimizing the management of ICI treated patients, considering that regular CT, MRI and PET scans are routinely performed in the course of an individual's cancer treatment. This diagnostic information should be able to be fed back to clinicians in a timely fashion resulting in the earlier identification and management of endocrine dysfunction than what currently occurs.

Finally, these data highlight the importance of long term follow up in a specialist endocrine clinic once the diagnosis of an endocrinopathy associated with ICI therapy is established, to

ensure optimal patient education, replacement of deficient hormones, and the completion of appropriate complication screening. At our centre, the findings that have emanated from my thesis have been presented to oncology, radiology and endocrinology groups to discuss translation of the recommendations to clinical practice. Ultimately, this work has contributed to a wider adoption of a systematic approach to the surveillance and management of endocrine irAEs in patients treated with checkpoint inhibitors.

5.3 Future directions

Strategies for prediction and early detection of these iatrogenic endocrine diseases would be of great benefit to patients undergoing ICI therapy. The presence of islet and thyroid autoantibodies before treatment with PD1 inhibitors is still not well described and it is not known what proportion of these irAEs occur in patients with existing antibodies or if new antibody seroconversion leads to *de novo* islet autoimmunity. The next phase of my research is to evaluate the prevalence of detectable islet and thyroid autoantibodies at baseline and after treatment with immune checkpoint inhibitors in a cohort of patients with melanoma. This study will utilise stored biospecimens from before and after treatment with CTLA-4 and PD-1 based regimens in 200 patients with melanoma as collected in the Biomarker Study (Principal Investigator Dr Shahneen Sandhu). Autoantibodies will be measured at three time points; baseline (pre-treatment), 3 months post first infusion and 6 months post first infusion. Routine biochemical data including TFTs, glucose, HbA1c and lipase will be extracted from the online medical records. Clinical diagnoses of thyroiditis, new onset diabetes, dysglycaemia or autoimmune pancreatitis will be obtained from a retrospective chart review. Data will be stored in a newly designed REDCap database based on the data dictionary designed for this thesis.

This collaborative effort is the first step towards the formation of a comprehensive biobank storing biospecimens from baseline and during treatment with ICI. Access to serum, peripheral blood mononuclear cells (PBMCs), tumour tissue and other tissue such as stool/microbiome would allow for a vast array of research opportunities, ultimately aimed at improving patient outcomes.

The development of a novel pituitary autoantibody assay is of significant interest. I have commenced discussions with key stakeholders at local clinical and research centres to develop a research group dedicated to this task.

Strategies to prevent or reverse endocrine toxicity, particularly diabetes and hypophysitis have not yet been studied prospectively. Our findings suggest there may be a narrow window of opportunity to reverse islet cell or pituitary dysfunction when detected early and imaging may be the mechanism with which this early detection could occur. As such, there may be a role for high-dose glucocorticoids in patients with imaging evidence of pancreatic or pituitary inflammation who have not yet developed gland failure. Given the prevalence of hypophysitis after cICI, this could feasibly be evaluated in a randomized controlled trial incorporating early pituitary MRI and/or FDG-PET/CT. Studies of biologic agents such as inhibitors of TNF-alpha or the JAK1/JAK/2 pathway in ICI related diabetes are underway in the preclinical setting and if successful may lead to significant translational advances.

Information gathered from such studies may provide insight into spontaneous autoimmune endocrinopathies and may add to the work being done in diabetes prevention studies in Australia. Understanding underlying mechanisms of activation of autoimmunity by checkpoint inhibitors may in addition, help in developing agents to target therapeutic

induction of exhaustion of auto-reactive T cells as a pertinent treatment approach for autoimmunity.

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Appendices

Appendix 1. Australasian Diabetes Congress Poster: Classic and non-classic presentations of immunotherapy related type 1 diabetes

Appendix 2. Manuscript: High dose glucocorticoids masking immune checkpoint inhibitor diabetes mellitus in two patients treated for metastatic melanoma

Appendix 3. Pictorial essay: The imaging of immunotherapy-related hypophysitis and other pituitary lesions in oncology patients

Classic and non-classic presentations of immunotherapy related type 1 diabetes

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INTRODUCTION

Immune checkpoint inhibition (ICI) has become standard of care in the management of many solid organ and haematological malignancies. The unprecedented survival benefit has been offset by the occurrence of collateral immune damage to healthy tissues, termed immune related adverse events (irAEs).

Diabetes associated with inhibition of programmed death protein – 1 (PD-1) or it's ligand PDL-1 occurs in around 0.9% of patients and is emerging as a clinically significant treatment complication.

Since initial published reports describing fulminant diabetic ketoacidosis (DKA) in 2015, a spectrum of diabetes presentations has become evident. In the move to search for a therapeutic intervention, understanding the type of diabetes is imperative.

METHODS

We reviewed the published literature to establish the common features of checkpoint inhibitor related diabetes.

On review of local cases between 2015-2019, we identified three patients diagnosed with checkpoint inhibitor related diabetes who presented with atypical features.

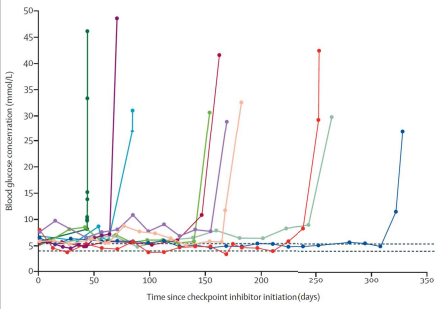
Relevant clinical, biochemical and radiographical evidence was examined and compared to the classic presentation described in the literature.

CLASSIC CHECKPOINT INHIBITOR ASSOCIATED DIABETES

In three recently published case series (1-3) which included 73 patients, we have observed a phenotype of accelerated and fulminant islet autoimmunity characterised by the following features:

1. Acute, symptomatic rise in plasma glucose concentration with relatively low HbA1c at presentation suggesting rapid onset
2. Median time of onset from initiation of checkpoint inhibitors of 6 weeks (although the range was wide: 1–228)
3. C peptide was suppressed or undetectable in more than 90%
4. Anti-glutamic acid decarboxylase (GAD) autoantibody was present in approximately half. Approximately 62% expressed the high-risk class II HLA allele, HLA-DR4

Figure 1. Progression to hyperglycaemia in patients with checkpoint inhibitor-induced diabetes (Perdigoto et al.)



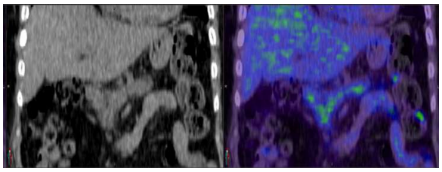
Glucose trends over time for ten patients whose clinical course was reported previously.

At present, there are no prospective studies investigating a drug for reversal of β-cell destruction following ICI. Glucocorticoids have not been effective and permanent insulin dependent diabetes follows (1). Recently, a report was published describing reversal of ICI related diabetes with the TNF-alpha inhibitor, infliximab (5). In this case, confounding factors such as recent intra-articular steroid use and evidence of impaired insulin sensitivity raise doubt over the response mechanism. Whilst it is timely to consider immune therapies for this iatrogenic form of diabetes, we must first be sure the patient has autoimmune diabetes.

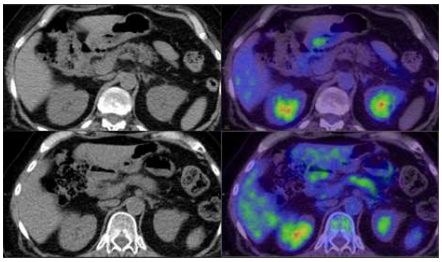
NON-CLASSIC PRESENTATIONS

CASE 1	
Age / Sex	65 / F
Past history	Coeliac Disease Psoriasis Lupus anticoagulant + Hypogammaglobulinaemia
Tumour type	Relapsed lymphoma following autologous stem cell transplant
Treatment	PD-1 inhibitor (Tislelizumab) + BTK inhibitor (Zanubrutinib)
Time to onset	30 months
Diabetes presentation	Admitted with severe oligoarthritis Increased pancreatic avidity noted on F-18 FDG PET

Figure 2. F-18 FDG PET



2a Coronal images show diffuse uptake in the pancreas



2b Compared to baseline imaging, the pancreas has become small and amorphous

Initial biochemistry	Glucose 11.8 mmol/L C peptide 0.79 nmol/L Lipase 12 U/L (8-78) HbA1c 59 nmol/L (7.6%) GAD< 5 U/ml IA2 < 0.3 U/ml HLA not known
Progress	Target glycaemia achieved with metformin 1g daily and gliclazide 40mg daily (HbA1c 6.7% at follow up) GAD seroconversion noted 4 months following presentation: - GAD Ab 42 IU/ml (<10) - IA2 Ab and ZnT8 remain undetectable - C peptide 0.61 nmol/L (0.4-1.5)
Impression	Subacute autoimmune diabetes possible (Increased FDG avidity in the pancreas and GAD seroconversion) however C peptide remains detectable.

CASE 2	
Age / Sex	65 / M
Past history	Type 2 diabetes, stable glycaemia on metformin, gliclazide and empagliflozin
Tumour type	Melanoma
Treatment	PD-1 inhibitor (Pembrolizumab)
Time to onset	2 days
Diabetes presentation	DKA
Initial biochemistry	Glucose 24 mmol/L Serum ketones 6 mmol/L pH 7.24 (7.35-7.45) C peptide 0.38 nmol/L HbA1c 57 mmol/mol (7.4%) GAD Ab <0.6 U/ml IA2 Ab <0.3 U/ml HLA not known
Progress	Rapid deterioration resulting in palliation
Impression	The contribution of the SGLT2 inhibitor is unknown. Early autoimmune diabetes with accelerated ketosis is possible.

CASE 3	
Age / Sex	65 / M
Past history	Hypertension
Tumour type	Melanoma
Treatment	PD-1 inhibitor (Nivolumab) + CTLA4 inhibitor (Ipilimumab)
Time to onset	19 months
Diabetes presentation	Presented with DKA on a background of 3 weeks of polyuria, polydipsia and weight loss.
Recent background	Completed an eight week course of weening prednisolone for pneumonitis two months prior to diabetes presentation. A random blood glucose was 6.6mmol/L two weeks before symptom onset.
Initial biochemistry	Glucose 21.5 mol/L B hydroxy butyrate 5.2 mmol/L (<0.3) pH 7.29 (7.35-7.45) HCO3 23 mmol/L (22-30) pCO2 47 mmol/L (35-45) C peptide 0.3 nmol/L (0.26-1.73) HbA1c 105 nmol/L (11.8%) GAD< 5 U/ml IA2 < 0.3 U/ml ZnT8 Ab < 10 U/ml HLA DR3/DR4 not expressed
Impression	Ketosis at presentation was associated with a respiratory acidosis and a normal serum bicarbonate, atypical for DKA. The c peptide was detectable, antibodies negative, and no high-risk class II HLA allele detected.

DISCUSSION

Unlike spontaneous type 1 diabetes, PD-1 inhibitors most often induce a rapid islet failure suggesting a narrow window for therapeutic intervention. The occurrence of islet antibody seroconversion can be seen in approximately 50% of cases. Elevation in GAD autoantibodies before treatment is also reported (1), indicating that ICI may both induce de novo autoimmunity and reinvigorate an existing dormant or exhausted immune response. HLA DR4 is expressed in the majority of patients, with an incidence higher than that seen in spontaneous diabetes [62% versus 43%] (4).

We suggest three diabetes phenotypes that may been seen after ICI:

1. **Classic autoimmune diabetes:** Early onset, acute hyperglycaemia with islet autoantibodies
2. **Subacute autoimmune diabetes:** Insidious presentation months after commencing ICI. Seroconversion eventually occurs and C peptide is low (case 1)
3. **Non-autoimmune diabetes:** Hyperglycaemia may be associated with other factors such as pancreatic metastases, glucocorticoid induced diabetes or exocrine pancreatitis. SGLT-2 inhibition in this setting may explain ketosis (cases 2 and 3)

Reversal of this process would significantly improve disease burden. Immunomodulation could be considered in cases with evidence of acute or subacute islet autoimmunity.

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High Dose Glucocorticoids Masking Immune Checkpoint Inhibitor Diabetes Mellitus in Two Patients Treated for Metastatic Melanoma

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Keywords: autoimmune pancreatitis**,** autoimmune diabetes mellitus**,** immune-related adverse events, glucocorticoid-induced diabetes.

Declarations:

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List of Abbreviations:

Immune checkpoint inhibitor induced diabetes mellitus (CPI-DM)

glucocorticoid induced diabetes (GID)

immune-related adverse events (irAEs)

Immune checkpoint inhibitors (CPIs)

diabetic ketoacidosis (DKA)

Common Terminology Criteria for Adverse Events (CTCAE)

positron emission tomography with 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG-PET)

glutamic acid decarboxylase (GAD)

programmed cell death protein -1 (PD-1)

cytotoxic T-lymphocyte-associated protein 4 (CTLA4)

Abstract

Background: Immune checkpoint inhibitor induced diabetes mellitus (CPI-DM) is a rare but life-threatening immune-related endocrinopathy, while glucocorticoid induced diabetes (GID) is a more frequent cause of hyperglycemia in the setting of glucocorticoid responsive immune-related adverse events (irAEs). CPI-DM diagnosed during concurrent CPI and high-dose glucocorticoid treatment has not been reported, and GID can mask or delay the diagnosis of CPI-DM. There are potential pathophysiological mechanisms for synergistic destruction of beta islet cells with these agents.

Cases presentation: We report two cases of metastatic melanoma with CPI-DM diagnosed in the context of dual CPI and high-dose glucocorticoid therapy. Case 1 was initially diagnosed as GID after 2 cycles of ipilimumab and nivolumab when the patient developed enterocolitis requiring glucocorticoid treatment. The diagnosis of CPI-DM was subsequently established with recurrent diabetic ketosis when insulin was withheld. Case 2 had pancreatitis evident on FDG-PET imaging but remained well for 6 months until high dose dexamethasone was commenced for cerebral metastasis. Severe diabetic ketoacidosis occurred after 6 weeks, requiring insulin therapy, and was associated with marked pancreatic atrophy. Glucocorticoid and glucose toxicity might have exacerbated underlying autoimmune pancreatitis.

Conclusion: These cases of dual CPI and glucocorticoid therapy confirm that high dose glucocorticoids do not reverse CPI-DM. Glucocorticoids may accelerate CPI-mediated islet cell destruction and can mask the diagnosis of CPI-DM which, if delayed, has serious consequences.

Background

Immune checkpoint inhibitors (CPIs) have revolutionised cancer therapy, with improved overall survival and progression-free survival in some previously treatment-refractory malignancies [1-3]. The efficacy of CPIs is offset by a myriad of immune-related adverse events (irAEs), which includes the rare but life threatening endocrinopathy termed immune checkpoint inhibitor induced diabetes mellitus (CPI-DM). CPI-DM leads to abrupt onset symptomatic hyperglycemia associated with severe insulin deficiency, characterised by a low or absent C-peptide and relatively low HbA1c at presentation[4]. Approximately three-quarters of patients present with diabetic ketoacidosis (DKA), which is associated with a high risk of multi-organ failure and death[4]. While CPI-DM is rare, new onset hyperglycemia is frequently seen in patients receiving high-dose glucocorticoids for other irAEs. In addition to insulin resistance induced by glucocorticoids, glucose toxicity is known to exert deleterious effects on beta-cell function[5],and glucocorticoid induced hyperglycemia may expedite islet destruction triggered by CPI. High-dose prednisolone treatment (2 mg/kg) in a case of pembrolizumab associated DKA did not reverse CPI-DM [6]. Here we report two cases of CPI-DM who presented with diabetic ketosis shortly after commencement of concurrent high-dose glucocorticoids.

CASE 1

A 78 year-old female with metastatic BRAF F583Y mutant melanoma involving lung, liver, abdominal wall and retroperitoneum was commenced on combined ipilimumab and nivolumab. She had normoglycemia prior to CPI therapy with a HbA1c of 31.1 mmol/mol (5.0%). Her second cycle was complicated by Common Terminology Criteria for Adverse

Events (CTCAE) Grade 3 enterocolitis[7], managed with 1mg/kg oral prednisolone, and thyroiditis (TSH 0.01 mU/L [RR 0.35-4.94], T4 30.8 pmol/L [RR 9.0-19.0]) (Fig. 1a). She represented within one week of glucocorticoid initiation with polyuria, polydipsia and urinary incontinence. Her admission blood glucose was elevated at 40 mmol/L (CTCAE Grade 4) with elevated plasma ketones of 3.7mmol/L [RR <1.5] and normal acid base status (Table 1). Hyperglycemia was managed with a basal bolus regimen of isophane and insulin aspart. During admission, insulin was withheld while fasting for a positron emission tomography with 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG-PET) scan, which did not reveal pancreatic uptake of FDG. Metabolic parameters rapidly deteriorated when insulin was withheld, with development of hyperglycemia (glucose 31 mmol/L) and ketosis (ketones 2.1 mmol/L). Lipase and faecal elastase were both normal, excluding exocrine insufficiency. A paired C-peptide was low at 0.16 nmol/L [RR 0.26-1.73] in the setting of elevated autoantibodies to glutamic acid decarboxylase (GAD) consistent with pancreatic insulin deficiency and CPI-DM (Table 1).

CASE 2

A male in his early 70s with progressive metastatic BRAF V600K mutant melanoma to brain, lung, liver and muscle despite dabrafenib and trametinib therapy was switched to dual CPI therapy with ipilimumab and nivolumab. After one cycle of CPI therapy, he developed enterocolitis requiring a single dose of 5mg/kg infliximab and pulse methyl prednisolone therapy (Figure 1b). He was normoglycemic prior to CPI therapy with a HbA1c of 38.8 mmol/mol (5.7%) and remained normoglycemic 5 months after dual CPI therapy. After cycle 1 of maintenance nivolumab, a routine surveillance FDG-PET demonstrated incidental

finding of increased FDG uptake in the pancreas (Figure 2f). Concurrently, pancreatic enzymes were elevated (lipase 489 U/L [RR 8 -78], amylase 150 U/L [RR 22-100]) and fasting glucose was 7.6mmol/L. Subsequent random glucose levels varied between 7 to 10.9 mmol/L without treatment. Due to progressive intracranial disease 6 months later, vemurafenib and cobimetinib were introduced with 8mg oral dexamethasone twice daily for cerebral oedema (Figure 1b). Within 6 weeks of glucocorticoid commencement, the patient presented with dizziness and subsequently was diagnosed with DKA requiring Intensive Care admission. His low c-peptide was consistent with pancreatic beta islet cell deficiency (Table 1). Subsequent imaging confirmed the development of significant pancreatic atrophy over time. (Figure 2c,d)

DISCUSSION

Immune checkpoint inhibitor induced diabetes mellitus is a rare irAE characterised by accelerated, fulminant islet cell destruction, most commonly reported with programmed cell death protein -1 (PD-1) inhibitors [8]. The incidence is estimated between 0.2 and 1.4% [4] , as compared to the more common endocrinologic irAE of thyroiditis at 33% [9]. GID, on the other hand, is a common cause of hyperglycemia in the oncology population. Short-term glucose excursion into the diabetic range was noted in up to 43% in patients receiving glucocorticoids for central nervous system metastasis, chemotherapy, radiotherapy or bone marrow transplantation [10, 11] .High-dose glucocorticoids are often required in non-endocrine irAEs and the treatment duration can be protracted[12]. As the likelihood of GID in a patient presenting with new onset glycemia is much greater, the presence of high-dose glucocorticoids may distract from recognition of underlying islet autoimmunity. A low index

of suspicion for CPI-DM is required in patients treated with CPI to avoid delays in more intensive insulin treatment. The presence of ketosis or ketoacidosis and a low C-peptide should prompt evaluation of islet autoantibodies and consideration of this diagnosis.

While elevated islet autoantibodies are seen in >90% of children with spontaneous Type 1 Diabetes, only 50% of patients with CPI-DM have detectable antibodies at presentation[4]. A systematic review of 71 cases of CPI-DM found that subjects with elevated islet antibodies at onset of CPI-DM were more likely to present early and with more severe diabetic ketoacidosis[8]. While it is unknown whether pre-treatment titre assists to triage risk for CPI-DM[13], high risk class II HLA alleles (DR3, DR4, DQ2 and DQ8) were found in 85% of patients with CPI-DM who underwent HLA typing[14]. Whether PD-1 blockade initiates de novo diabetes or accelerates islet autoimmunity in people with existing autoantibodies or a genetic predisposition to diabetes has not been prospectively evaluated[15].

We report two cases where the presentation of CPI-DM occurred shortly after initiation of high-dose glucocorticoids. In both cases, a normal HbA1c at baseline excluded the presence of pre-existing diabetes. In case 1, withdrawal of insulin for an FDG-PET scan unmasked a severe insulin deficiency and prompted evaluation of islet autoantibodies, which were significantly elevated. In case 2, a suspected sub-acute irAE pancreatitis (as evidence by increased pancreatic uptake of FDG, asymptomatic rise in the pancreatic enzymes, and elevated fasting glucose) progressed to diabetic ketoacidosis 6 months later, following the initiation of high-dose dexamethasone. Subsequent pancreatic imaging demonstrated marked pancreatic atrophy (Figure 2). Similar loss of pancreatic volume can be seen in children and adolescents in the first 12 months after diagnosis of spontaneous Type 1 Diabetes. The mechanism of pancreatic atrophy is not fully understood, but may relate in

part to the fulminant destructive insulitis and reduction of B cell mass with resultant loss of trophic effect of insulin on acinar cells[16]. Diffuse uptake of FDG in the pancreas has been described in patients with endocrine and exocrine pancreatic insufficiency after CPI, but is not seen in all cases of CPI-DM[17]. Biochemical markers of pancreatic injury can be elevated in the setting of immunotherapy, with a meta-analyses of CPI clinical trials suggesting rates of lipase elevation in approximately 2.7% of patients [18] or a relative risk of 1.5 [19]. In both of these meta-analyses, the risk was higher with cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or dual CTLA4/PD-1 inhibition compared with PD-1 inhibitors alone. The clinical significance of the lipase elevation in this study is unclear since the risk of any grade pancreatitis with CPI use was comparable to controls subjects[19]. Due to the limitations of clinical trial data used in these meta-analyses, correlations of CPI-induced lipase/pancreatitis rates with new onset DM remain unknown.

Most irAEs develop within weeks to months after CPI initiation[20]. Our centre has previously described 9 cases of CPI-DM, all of whom presented within 12 weeks of CPI commencement [15]. The onset of hyperglycaemia in our two subjects was preceded by a short course of high-dose glucocorticoid. The association of high-dose glucocorticoid use with CPI-DM development has not been previously reported. However, the concept of glucocorticoid toxicity to the pancreatic islet cells is well described in the islet transplantation literature [21, 22], and alongside the glucose toxicity may present a double jeopardy to the CPI compromised islet cells. Glucocorticoids increase peripheral insulin resistance, coercing pancreatic islet cells to increase insulin production. Chronic exposure of rodent islets to dexamethasone leads to decreased glucose-stimulated insulin secretion and reduction in insulin secreting cell lines[23]. PD-1 inhibition leads to re-invigoration of

exhausted T cells, unleashing an immune response which can manifest as pancreatitis. PD-L1 expression has been reported in Non-obese diabetic mice during autoimmune diabetes. Presumably, PD-1 blockade may accelerate autoimmunity in a patient with a genetic predisposition being kept “in-check” by inhibitory checkpoint receptors [24].

The presentation of CPI-DM can be heterogenous, encompassing the milder diabetic ketosis as seen in Case 1, as well as the more severe diabetic ketoacidosis as described in Case 2. A third scenario, non-ketotic hyperglycemia, has also been reported in 17% of CPI-DM in case series[25]. It is essential to distinguish GID from CPI-DM, as the latter will not improve on withdrawal of steroids and unrecognised insulin deficiency can lead to catastrophic consequences. The consensus recommendations for management of CPI-DM by the Society for Immunotherapy of Cancer Toxicity Management Working Group suggest baseline Hba1c and glucose should be checked prior to CPI commencement[7]. However, the range of fasting glucose seen in CTCAE Grade 2 and above autoimmune diabetes (glucose > 8.9 mmol/L or 160 mg/dL) overlaps with glucose levels seen in patients on glucocorticoid treatment. C-peptide is often checked late in the presentation of established ketosis to differentiate between GID and CPI-DM. To date, there are no guidelines regarding the monitoring pathway of patients on CPI and glucocorticoids. It is unknown whether protracted low dose versus short term high dose glucocorticoids in these subjects contributes to the development of CPI-DM. Further studies are required to establish whether the rate of CPI-DM is higher in the presence of concurrent high dose glucocorticoids, and to establish a glucose monitoring regimen to avoid unexpected DKA.

CONCLUSION

CPI-DM is a rare but potentially lethal irAE. The concurrent use of high-dose glucocorticoids may accelerate CPI induced islet cell destruction and may also mask this diagnosis, therefore delaying appropriate insulin therapy. Development of clinical pathways to monitor glucose and ketone levels in these patients are crucial to facilitate early diagnosis and intervention.

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Table 1: Biochemistry results at the time of autoimmune diabetic ketosis presentation.

Analyte	Case 1	Case 2	Reference Interval
Glucose	40	26	3.3 – 7.7 mmol/L
Ketone	3.7	6.8	< 1.5 mmol/L
pH	7.4	7.23	7.35 – 7.45
C-peptide	0.16	0.3	0.26 – 1.73 nmol/L
Pre-treatment HbA1c	31.1 mmol/mol (5%)	38.8 mmol/mol (5.7%)	< 47.5 mmol/mol (<6.5%)
Admission HbA1c	Not available	10.4	< 6.5%
GAD Ab	>2000	< 5	< 5 U/ml
IA2 Ab	< 7.5	< 7.5	< 7.5 U/ml
ZnT8 Ab	< 10	< 10	< 10 U/ml

Figure 1: Timeline of CPI and glucocorticoid use prior to diabetic ketosis in a) Case 1 and b) Case2.

Fig. 1a

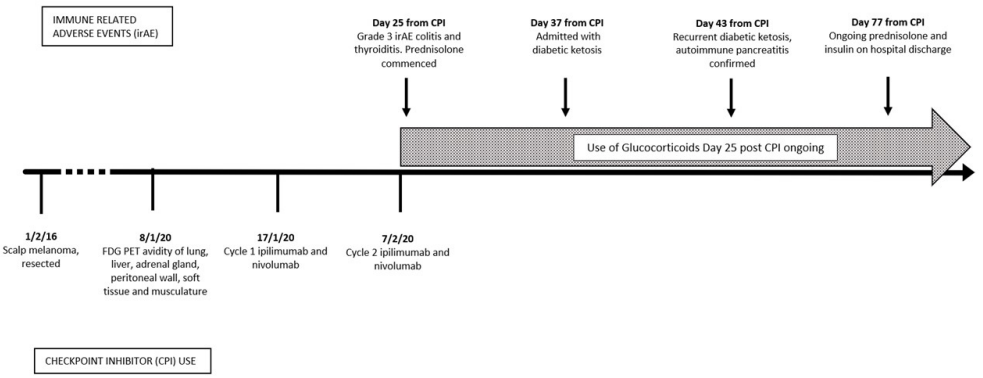

Fig. 1b

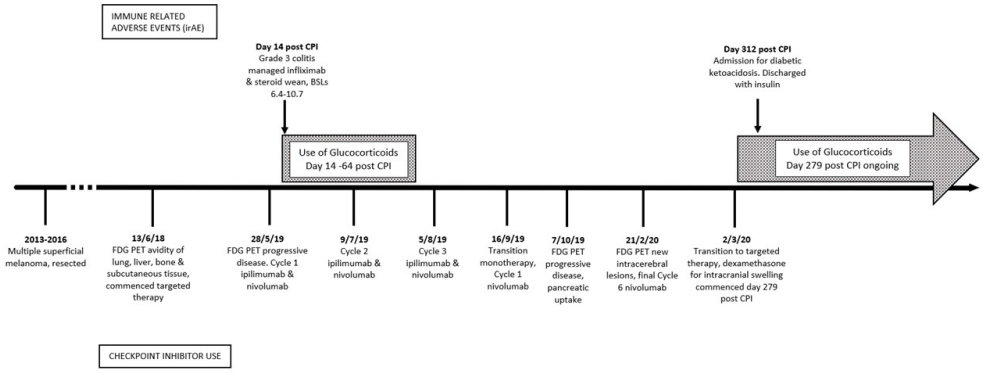

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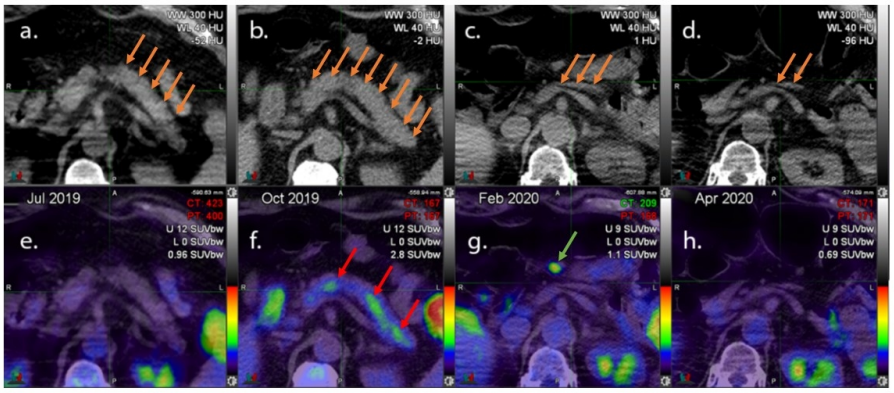
Figure 2: Serial non-contrast CT (a-d) and fused FDG PET/CT (e-h) from baseline evaluation through to late follow-up demonstrates initial enlargement followed by atrophy of the pancreas (orange arrows). The first post-treatment scan demonstrated patchy increased uptake in the pancreas (red arrows), which subsequently resolved. Transient uptake that developed subsequently in an adjacent peri-pancreatic node (green arrow) is felt likely to relate to immunological priming in a draining lymph node with resolution at later evaluation (pseudo-progression).



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Pictorial Review

The imaging of immunotherapy-related hypophysitis and other pituitary lesions in oncology patients

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Immunotherapy has revolutionised the treatment of metastatic disease from a variety of different primaries, but is frequently associated with immune-related adverse events. This review illustrates the imaging features of immunotherapy-related hypophysitis (IH) and some of the important differential diagnoses in oncology patients. The key radiological characteristic of IH is diffuse, modest enlargement of the pituitary gland with temporal evolution attributable to immunotherapy. Pituitary enlargement is transient, and the gland size returns to baseline size or smaller within months. IH is usually associated with homogeneous enhancement of the pituitary gland, and the pituitary stalk may be thickened. Larger pituitary size, deviation of the pituitary stalk, the presence of a discrete lesion surrounding by normal pituitary tissue, sellar expansion, and clival invasion are not typical of IH and suggest alternate diagnoses. On integrated 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron-emission tomography (PET)/computed tomography (CT), a transient increase in the metabolic activity of the pituitary gland with subsequent decline to background activity is also suggestive of IH. We suggest that the sella is assessed routinely on imaging performed in the first 6 months after commencing immunotherapy to detect subtle changes. Radiologists should also be aware of features that either support a diagnosis of IH or suggest alternate diagnoses.

Introduction

Immune checkpoint inhibitors (ICI), also known as immunotherapy, have revolutionised the treatment of metastatic disease from a variety of different primaries. These agents activate or re-invigorate the immune system's response to tumour cells, through blockade of inhibitory "checkpoint" receptors; however, enhancement of the immune system frequently results in bystander

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toxicity in the form of a variety of immune-related adverse events (irAEs). Immunotherapy-related hypophysitis (IH) is a commonly reported irAE and is a diagnosis for which imaging plays an important role. The propensity for IH differs between different ICI, and most commonly occurs with regimens containing cytotoxic T-lymphocyte-antigen 4 (CTLA-4) inhibition, in particular ipilimumab. Ipilimumab is most commonly used for the treatment of metastatic melanoma, either in combination with nivolumab or as monotherapy. This combination is also being increasingly used in other malignancies, including non-small cell lung cancer,[1] renal cell carcinoma,[2] and colorectal cancer.[3] IH occurs in 4–13% of patients treated with ipilimumab monotherapy, in contrast to <1% after single-agent programmed cell death protein 1 (PD-1) inhibition with pembrolizumab or nivolumab, while combination CTLA-4 and PD-1 inhibition (with ipilimumab and nivolumab) is associated with an incidence as high as 8–19%.[4–6] Hypophysitis outside the context of immunotherapy is rare, with an estimated annual incidence of only 1 in 7–9 million.[7] Histologically, hypophysitis may be classified as lymphocytic, granulomatous, xanthomatous, plasmacytic, or mixed.[7] Lymphocytic hypophysitis is the most common and has a greater incidence in females, related to an association with pregnancy.[7,8] In contrast, IH does not have a female preponderance; rather it may be more common in males and older patients.[7]

The clinical manifestations of IH relate to mass effect and/or pituitary hormone dysfunction.[7] Headache and fatigue are the most common presenting symptoms.[7,9] Visual symptoms are uncommon, as pituitary enlargement does not tend to compress the optic chiasm.[9] Anterior pituitary hormone deficiencies commonly involve the adrenal, thyroid, and gonadal axes. Diabetes insipidus is rare, occurring in only three of 60 (5%) patients with IH in one series,[10] while no cases were observed in three other series totalling 57 patients.[9,11,12] Incidental imaging evidence of IH may also be identified on routine follow-up imaging preceding an overt clinical syndrome.[5] In contrast, metastases are frequently associated with diabetes insipidus, with an incidence of 50% in a recent series.[10] Similarly, both anterior and posterior pituitary dysfunction are common in primary autoimmune (lymphocytic) hypophysitis, and panhypopituitarism may occur.[8] Primary autoimmune hypophysitis also has a tendency to produce greater enlargement of the pituitary (and thus a greater degree of local mass effect) than IH,[13] presumably due to the more prolonged and extensive T-cell infiltration.[7]

In the context of immunotherapy for metastatic disease, the key distinction is between IH and a metastasis. Incidental lesions are also added to the differential if no pre-treatment imaging is available, including adenoma, Rathke's cleft cyst, craniopharyngioma, and other causes of hypophysitis. The following review illustrates the imaging features of IH and some of the key differentials in oncology patients.

Typical magnetic resonance imaging appearances of hypophysitis

Magnetic resonance imaging (MRI) is the imaging technique of choice for hypophysitis. Computed tomography (CT) can also assess pituitary size, but other features of hypophysitis and potential differentials are not as readily assessable. When available, integrated 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron-emission tomography (PET)/computed tomography (CT) can provide complementary diagnostic information, but is not primarily used for the assessment of suspected IH. In the appropriate clinical context, an MRI diagnosis of IH can be made on routine sequences by craniocaudal measurement of the pituitary gland in the sagittal plane. Given the widespread use of volumetric contrast-enhanced T1-weighted imaging in the oncology setting, sagittal reconstructions are readily available, and these are adequate for diagnosing IH in most cases. Radiologists should routinely assess the pituitary gland, especially on imaging performed in the first 6 months after commencing ICI, and compare to baseline imaging as outlined below.

The key MRI feature of IH is diffuse, transient enlargement of the pituitary gland. In the coronal plane, this produces a tent-shaped or triangular appearance (Fig 1). Importantly, the pituitary enlargement is modest.[10,14] In a study containing 59 cases of IH, the peak absolute pituitary size was ≤2 cm in all cases and sometimes within the normal range.[10] This highlights the importance of comparison to pre-treatment baseline imaging[9] (Fig 2). In contrast, 74% of the 61 metastases in this series resulted in pituitary enlargement >2 cm.[10] Unlike the heterogeneous enhancement usually associated with pituitary metastases, hypophysitis is usually associated with homogeneous enhancement of the pituitary gland.[10] Thickening of the pituitary stalk often occurs with IH (59% of cases in one series),[10] but is not required to make the diagnosis. Indeed, metastases may also result in stalk thickening (28% of cases in the above series), limiting the discriminative value of this feature.[10] Isolated pituitary metastases are very rare and account for only 0.4% of all intracranial metastases.[15] Thus, the identification of other metastatic intracranial lesions does not necessarily indicate that the pituitary lesion is also a metastasis. Of further note, IH is most commonly encountered in the context of treatment of metastatic melanoma, as discussed above, but melanoma accounted for only 2% of all pituitary metastases in a recent systematic review (the most common primaries being lung and breast).[15]

The temporal evolution is critical for making the correct diagnosis, again highlighting the importance of pre-treatment neuroimaging. IH leads to a transient increase in pituitary size after ICI, which universally resolves within a few months (Fig 2). The final pituitary size may be smaller than the baseline size (Fig 2). Although this finding suggests pituitary destruction, the clinical and biochemical significance is unclear. In patients not

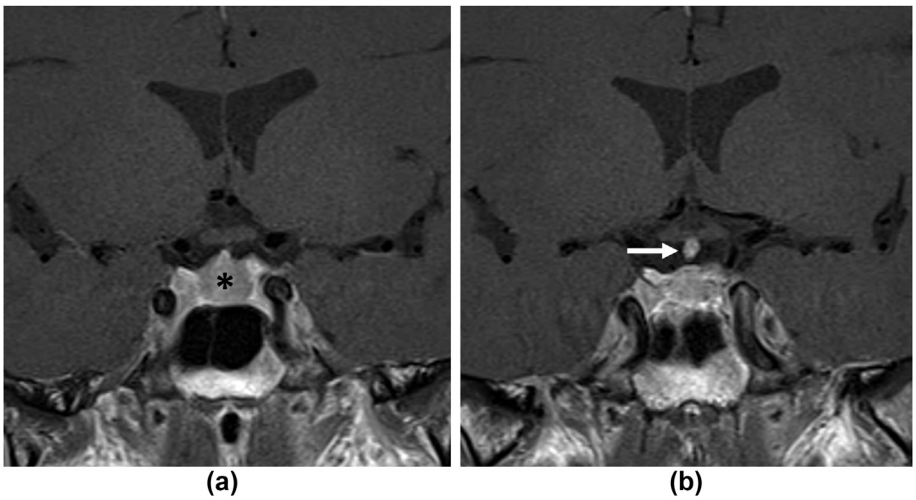

Figure 1 Contiguous coronal contrast-enhanced T1-weighted images of the pituitary gland in a patient with hypophysitis, demonstrating enlargement of the pituitary gland with a tent-shaped appearance (a, black asterisk) and thickening of the pituitary stalk (b, white arrow).

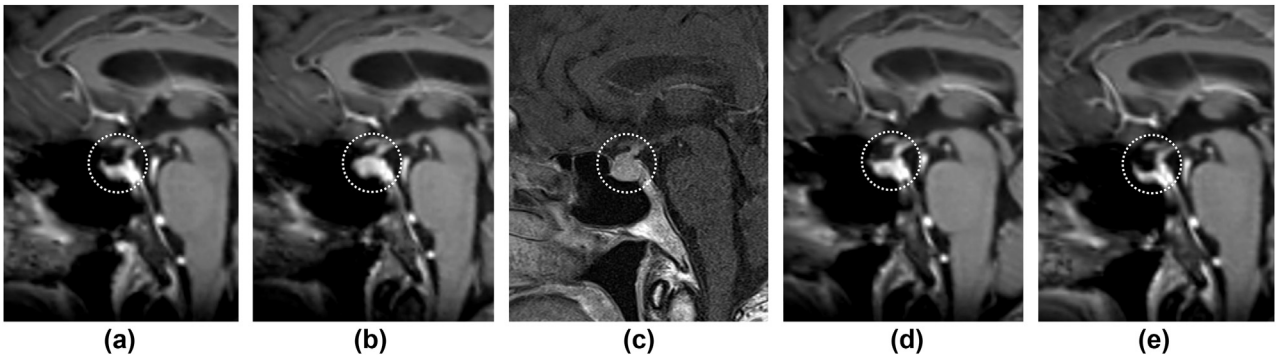

Figure 2 Evolution of the appearances of the pituitary gland in a patient who developed hypophysitis after combination immunotherapy, detected incidentally on routine follow-up imaging. (a) The pituitary gland is normal in size pre-treatment. (b) Routine follow-up imaging after immunotherapy showed mild but convincing enlargement, consistent with hypophysitis despite measuring within the normal range. At this stage, the patient was asymptomatic and serum cortisol was normal, though cortisol deficiency developed a short time later. (c) The pituitary gland enlarged further, before returning to a normal size after treatment (d). (e) On later follow-up imaging, the pituitary is smaller than on the pre-treatment baseline, raising the possibility of secondary hypopituitarism. (c,d) Corresponding evolution of pituitary stalk thickening.

already receiving pituitary hormone replacement, these changes should prompt longitudinal monitoring of pituitary hormone levels. In patients treated with glucocorticoids for concurrent irAEs, cautious withdrawal with reassessment of the pituitary–adrenal axis function may be required. If a pituitary abnormality was present prior to immunotherapy, alternate diagnoses must be considered. The evolution after commencing immunotherapy aids in the differentiation between a metastasis and an incidental lesion such as an adenoma, Rathke's cleft cyst or craniopharyngioma. A pituitary metastasis can be expected to increase or decrease in size in line with other intracranial metastases (Fig 3). In contrast, stability of the pituitary lesion despite clear progression or regression of metastatic disease elsewhere favours the diagnosis of a macroadenoma or Rathke's cleft cyst.

If the pituitary gland measures towards the upper limit of normal (≥7 mm) or appears subjectively bulky on neuroimaging performed within 3–6 months of commencing ICI, the current study should be compared to the pre-treatment baseline, in order to detect subtle enlargement. If pre-treatment imaging was not performed, it may be possible to later make a retrospective diagnosis of IH, based on a subsequent decrease in the size of the pituitary gland. Recognition of these subtle radiological findings should prompt longitudinal biochemical evaluation for evolving pituitary dysfunction.

Typical features of other pituitary lesions

The diagnosis of hypophysitis is more challenging if serial imaging is not available. IH typically produces only modest pituitary enlargement; thus, a larger (>2 cm in size) sellar mass is unlikely to be due to hypophysitis.[10] The epicentre of the lesion is important: in IH, the abnormality will predominantly affect the pituitary itself, with or without associated stalk thickening, while a larger suprasellar component indicates an alternate diagnosis, such as a

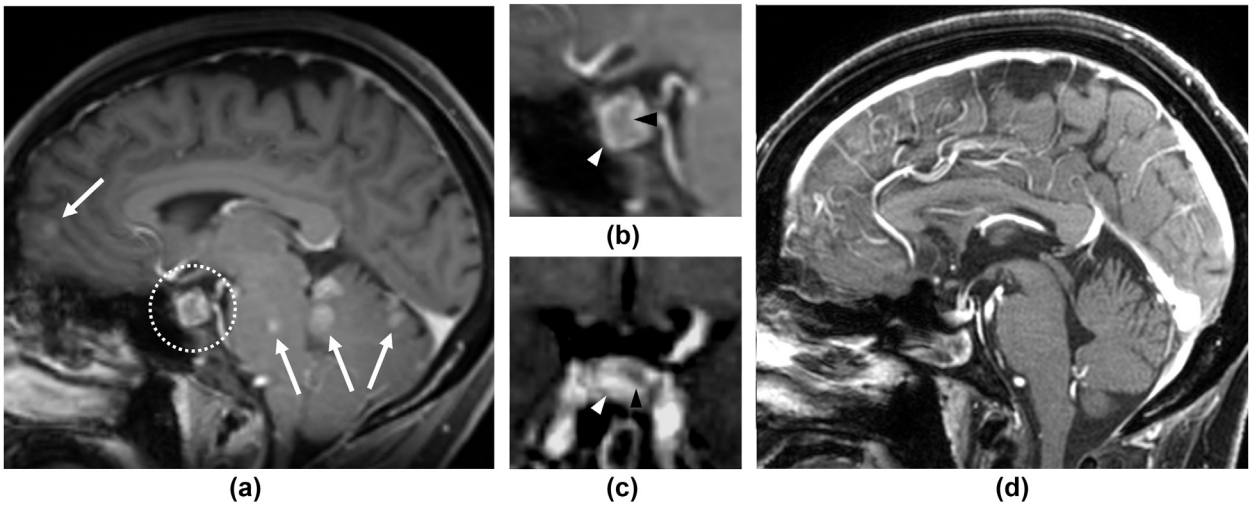

Figure 3 Contrast-enhanced T1-weighted images of a patient with metastatic breast cancer. (a) Before treatment there is a heterogeneous mass in the pituitary gland (dotted circle), as well as multiple metastases elsewhere intracranially (white arrows). (b) This heterogeneity is also demonstrated in the magnified sagittal and (c) coronal images, with the metastasis (black arrowheads) enhancing less avidly than the surrounding pituitary tissue (white arrowheads). (d) After treatment, interval resolution of the pituitary and other intracranial masses is consistent with a diagnosis of pituitary metastasis.

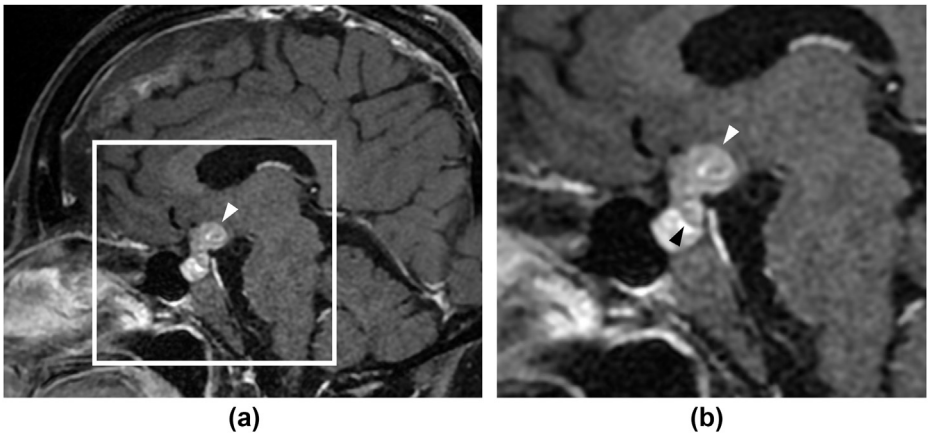

Figure 4 (a) Sagittal contrast-enhanced T1-weighted image, (b) including zoomed panel, of a pituitary region metastasis (white arrowheads), confirmed histologically. Although there is involvement of the pituitary gland, the epicentre of the lesion is suprasellar, and an interface (black arrowhead) is visible with the normal pituitary tissue (which enhances more avidly). This contrasts with hypophysitis (see Fig 2c), in which the abnormality is centred on the pituitary gland.

metastasis (Fig 4) or craniopharyngioma. Of note, craniopharyngiomas are typically suprasellar, and while they can involve the sella, only 5% of craniopharyngiomas are solely intrasellar.[16] IH does not invade surrounding structures such as the cavernous sinus; none of the 61 cases of IH in one series demonstrated cavernous sinus extension, while this was present in 51% of pituitary metastases.[10] The identification of a discrete lesion surrounded by normal pituitary tissue and deviation of the pituitary stalk are also atypical of hypophysitis[17] (Fig 5). Hypophysitis is a transient, acute process, and will not enlarge the sella. Generally, sellar expansion indicates an incidental chronic pituitary lesion, most commonly a benign macroadenoma. Rather than expanding the sella, metastases may invade through the sellar floor, and close assessment of the sellar floor for subtle invasion is worthwhile (Fig 6). A macroadenoma may also occasionally invade the clivus,[18] but metastasis and adenoma can usually be distinguished by their temporal evolution, as discussed above.

The role of FDG-PET/CT

FDG-PET/CT has particular value in the monitoring of both tumoural response and toxicities in patients receiving ICI.[19] The normal pituitary gland does not demonstrate FDG uptake above the background activity, thus any significant FDG uptake commonly indicates pathology (Fig 7). Due to physiological marked FDG uptake by the grey matter and potential spill over of activity by the adjacent cortex, particular attention needs to be made to this region on axial

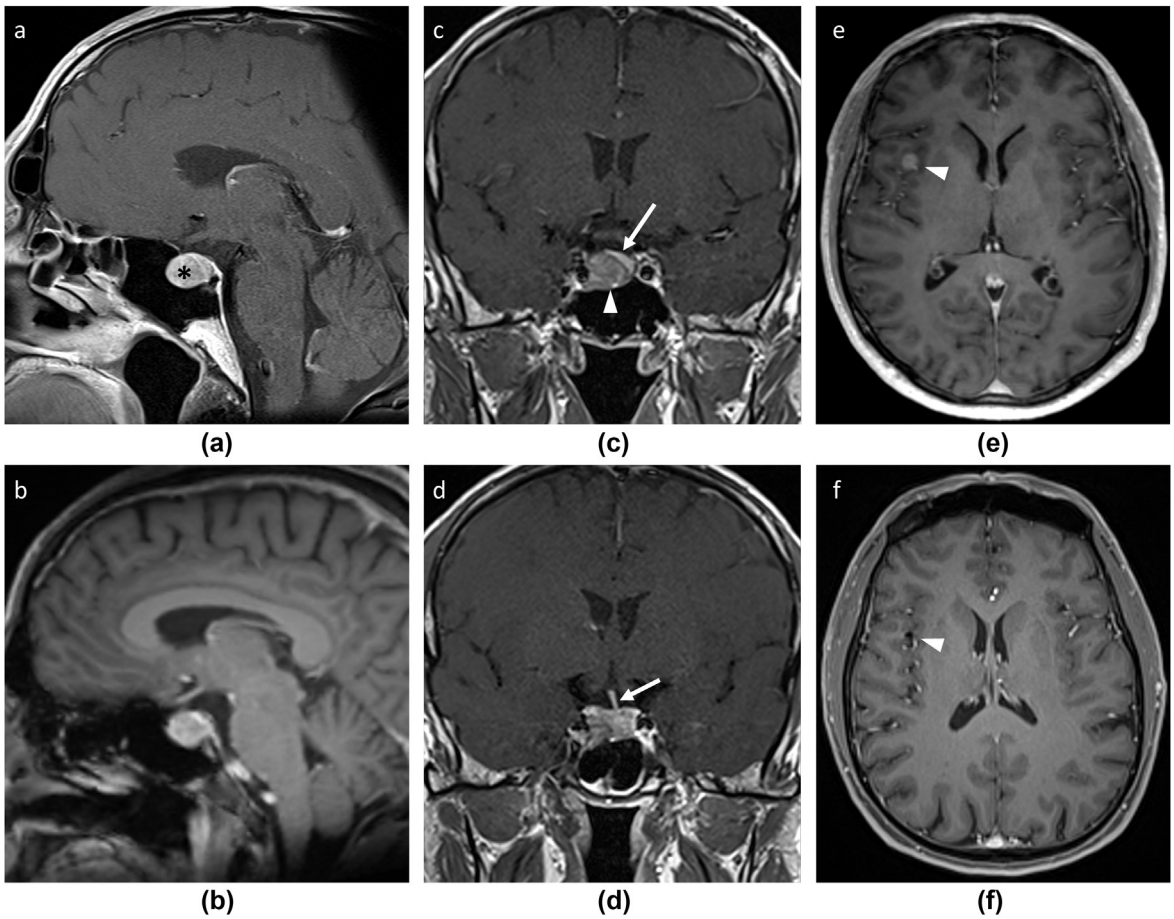

Figure 5 (a) Sagittal contrast-enhanced T1-weighted images in a patient with metastatic melanoma, demonstrating enlargement of the pituitary gland (12 mm craniocaudally) before immunotherapy (black asterisk), with no change almost a year after immunotherapy (b). (c) Coronal dynamic contrast-enhanced imaging shows the lesion (white arrowhead) to be distinct from the normal pituitary tissue (white arrow). (d) The pituitary stalk is displaced to the left (white arrow). The appearances and stability are consistent with an incidental macroadenoma. (e) Axial contrast-enhanced images show a right insular metastasis at baseline (white arrowhead), which essentially resolved after immunotherapy (f), with only residual low T1 signal (white arrowhead) due to blood products. Resolution of the intracranial metastasis with no change in the pituitary lesion further supports the diagnosis of an incidental pituitary macroadenoma.

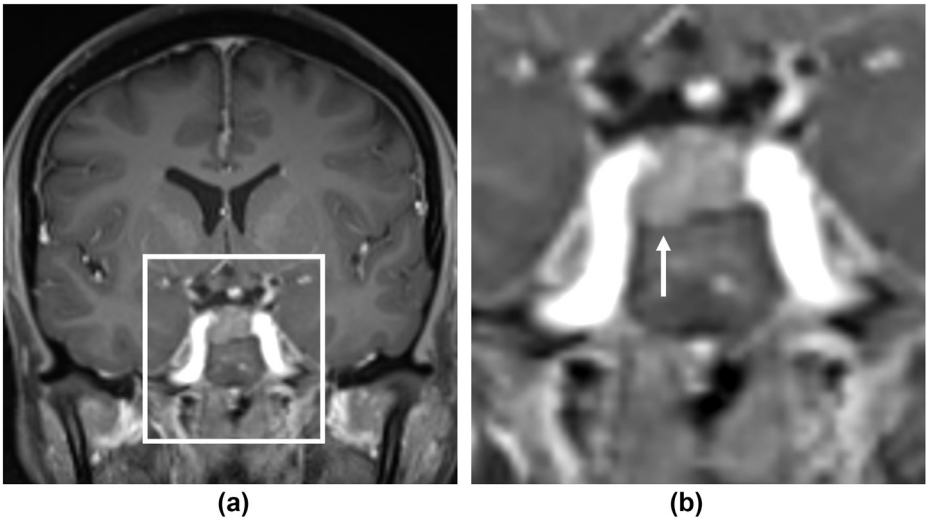

Figure 6 (a) Contrast-enhanced T1-weighted imaging in the coronal plane, including magnified panel (b), demonstrates a sellar mass with subtle invasion of the clivus (white arrow), consistent with a metastasis or macroadenoma rather than hypophysitis. (c,d) The clival invasion is also visible in the sagittal plane.

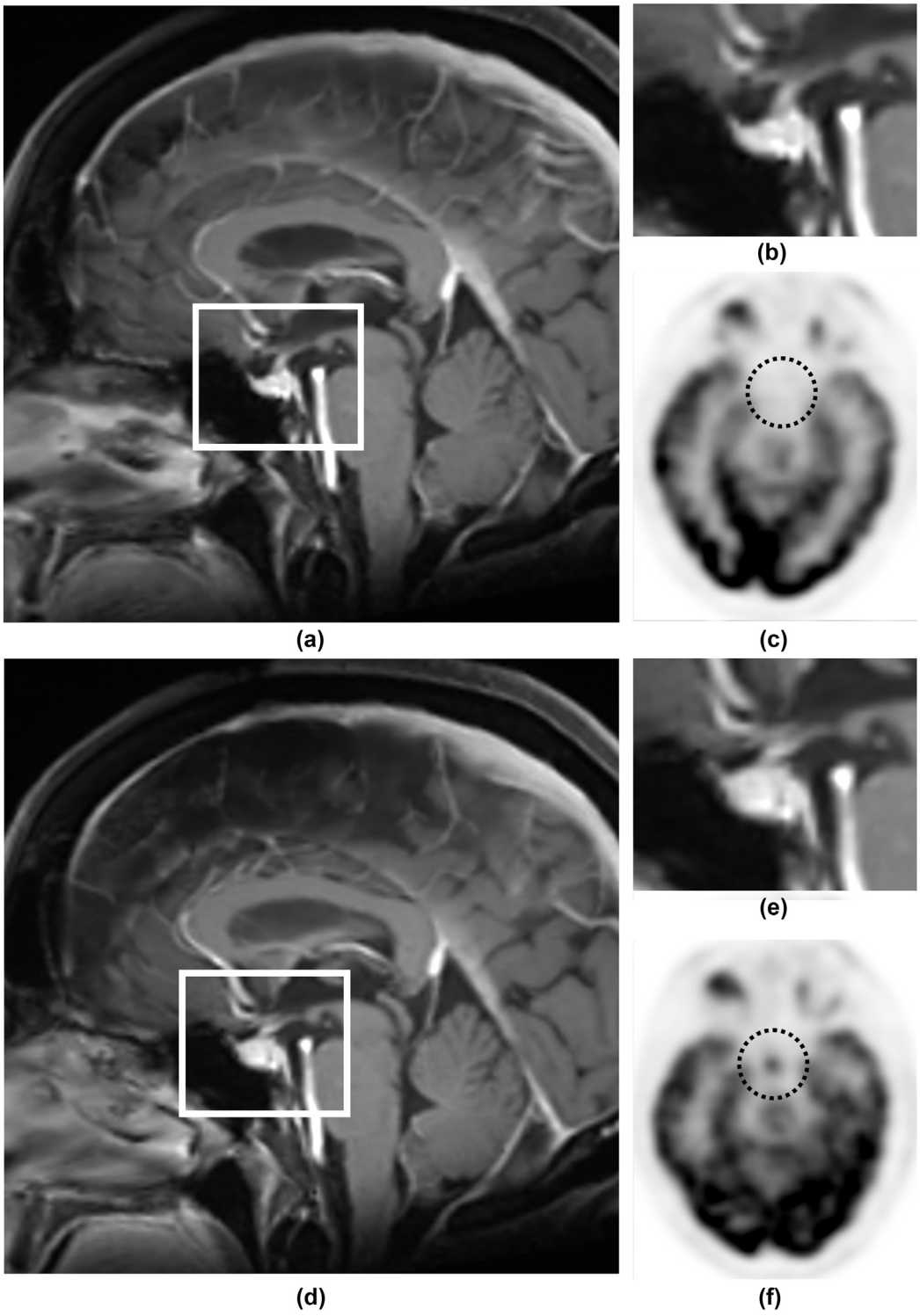

Figure 7 (a) Prior to ipilimumab monotherapy, the sagittal contrast-enhanced MRI image, including magnified panel (b), shows a normal pituitary gland measuring 6 mm craniocaudally, with no uptake on FDG-PET (c, dotted circle). (d,e) Although a subsequent craniocaudal pituitary measurement of 8 mm is within normal limits after ipilimumab, mild enlargement is appreciable compared to baseline. (f) New uptake on FDG-PET (dotted circle) further supports hypophysitis. The abnormal FDG uptake in the sella subsequently resolved (not shown).

images (either PET or fused PET/CT). We therefore recommend that the sella be assessed specifically on FDG-PET/CT studies performed routinely in the first few months after commencing ICI, in order to identify pathological uptake.

Without longitudinal imaging, PET cannot differentiate between hypophysitis and a neoplastic lesion such as a metastasis or macroadenoma.[20] Thus, as with MRI, the temporal evolution of FDG uptake in the sella in critical.

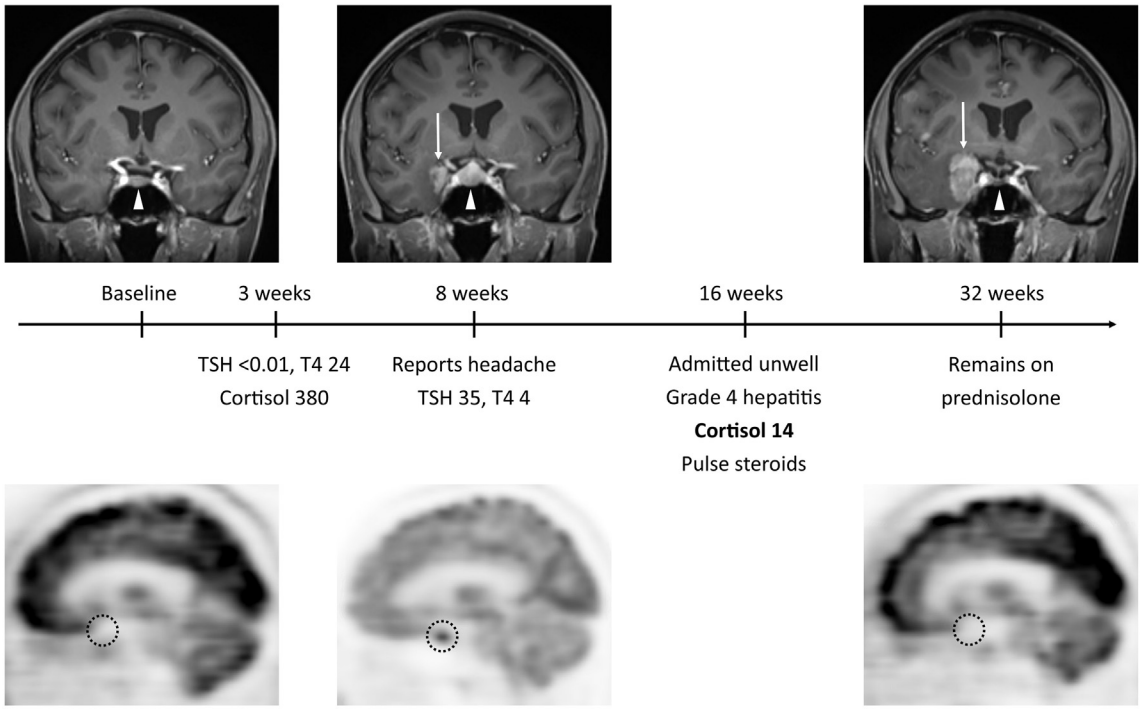

Figure 8 Evolution of imaging features of hypophysitis with clinical and biochemical correlation. Coronal MRI images (top panel) show enlargement of the pituitary gland from baseline with a typical tent-shaped appearance (arrowhead), at the time of onset of headache, but preceding the development of cortisol deficiency. The pituitary gland subsequently decreases in size to sub-baseline. The intracranial metastatic disease (best seen in the right temporal lobe, arrow) progresses over this period. Contemporaneous FDG-PET images in the sagittal plane (bottom panel) show abnormal FDG uptake in the sella (dotted circle) at the time of pituitary enlargement on MRI, with subsequent resolution.

New, transient pituitary FDG hypermetabolism after ICI correlates with IH.[5] A lack of FDG uptake in a sellar lesion is also useful for characterisation, suggesting non-neoplastic pathology such as a Rathke's cleft cyst or a microadenoma (approximately half do not demonstrate FDG uptake[20]). The information derived from the whole-body FDG-PET/CT can also provide further supportive evidence. For instance, new abnormal uptake in the sella in the presence of a favourable response of extracranial disease and/or the development of other FDG-PET/CT manifestations of irAEs would further support IH rather than a new pituitary metastasis.

Biochemical correlation

International guidelines recommend routine monitoring of electrolytes and thyroid function every 4–6 weeks in patients receiving ICI.[21] Formal anterior pituitary hormone evaluation of the thyroid, adrenal, and gonadal axes is performed in patients with evidence of new hyponatraemia, secondary hypothyroidism, or when there is a clinical suspicion. The clinical syndrome may be non-specific, and a high index of suspicion is required in patients with headache, lethargy, or malaise. Acute cortisol deficiency (fasting cortisol $<$250 nmol/l or a random cortisol $<$150 nmol/l) prompts urgent glucocorticoid replacement while awaiting the outcome of the remaining pituitary hormone levels. Radiological detection of pituitary enlargement may precede the clinical syndrome, however.[5] It follows that an incidental finding of pituitary enlargement should be considered an important prompt for biochemical evaluation for evolving pituitary hormone deficiency. Correlation between imaging, clinical and biochemical findings and their evolution is shown in Fig 8.

Concomitant irAEs are common in the first 6 months after ICI and may require prolonged and high-dose glucocorticoid administration. In this setting, symptoms of acute cortisol deficiency will be masked and insidious onset hypothyroidism or hypogonadism may go undetected. An imaging diagnosis of hypophysitis in these patients justifies close monitoring of pituitary hormones in order to detect and treat subclinical pituitary dysfunction.[5]

Conclusions

Immunotherapy-related hypophysitis is common, but the clinical diagnosis is challenging. We suggest that radiologists should specifically assess the pituitary gland on routine imaging performed within the first 6 months after commencing immunotherapy. If the gland size is $\geq$ 7 mm or appears bulky, comparison to baseline imaging is critical to appreciate the characteristic finding of subtle transient enlargement. Subsequent follow-up imaging will demonstrate normalisation to baseline or sub-baseline gland size. Similarly, a transient increase in FDG uptake in the sella is an important finding of IH. Radiologists should also be aware of features suggestive of alternate diagnoses, including metastases and benign incidental lesions.

Conflicts of interest

The authors declare no conflict of interest.

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