UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES

RETROSPECTIVE REVIEW OF WOMEN DIAGNOSED WITH PREMATURE OVARIAN INSUFFICIENCY

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CHRNYA002

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A dissertation submitted in partial fulfilment of the requirements for the Masters of Medicine degree (MMED) in Obstetrics and Gynaecology.

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Declaration by Applicant

I, **Nyatozi Leah Chirwa**, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being or is to be submitted for another degree in this or any other university.

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I supervised the research which Dr Nyatozi Chirwa has undertaken and the presentation of this dissertation.

I am satisfied that this is Dr Chirwa's original work and that this dissertation should be submitted as part of the requirements for the MMed (O&G).

Supervisor : Zephne M van Der Spuy

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List of Abbreviations

The following abbreviations are used throughout the dissertation

ACTH	Adrenocorticotrophic hormone
AMH	Anti-Mullerian Hormone
ASRM	American Society of Reproductive Medicine
BMP15	Bone morphogenic protein 15
BMD	Bone Mineral Density
CAH	Congenital adrenal hyperplasia
CG	Chorionic gonadotrophin
DHEAS	Dehydroepiandrosterone sulphate
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle Stimulating Hormone
FAI	Free androgen index
FMR	Fragile X mental retardation
FSHR	Follicle Stimulating Hormone receptor
FOXL2	Forkhead transcription factor
GALT	Galactose-1-phosphate uridyltransferase
GEC	Gynaecological Endocrine clinic
GnRH	Gonadotrophin releasing hormone
GSH	Groote Schuur Hospital

hCG	Human chorionic gonadotrophin
HT	Hormone therapy
IMS	International Menopause Society
LH	Luteinizing Hormone
NHLS	National Health Laboratory Services
NIH	National Institute of Health
PCOS	Polycystic ovary syndrome
POI	Premature ovarian Insufficiency
POD	Premature ovarian dysfunction
POF	Premature ovarian failure
PRL	Prolactin
SAMS	South African Menopause Society
SASOG	South African Society of Obstetricians and Gynaecologists
STAR	Steroidogenic acute regulatory protein
TSH	Thyroid stimulating hormone
USA	United States of America
UK	United Kingdom
WHI	Women's Health Initiative
WHO	World Health Organization

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Abstract

Premature ovarian insufficiency (POI) is a clinical syndrome defined by loss of ovarian activity before the age of 40. It is characterized by menstrual disturbance (amenorrhoea or oligo-amenorrhoea), raised gonadotrophin concentrations and low oestradiol levels. The diagnosis is confirmed by detection of raised serum follicle stimulating hormone and low oestradiol levels. POI can occur spontaneously, but it may also result from genetic defects, chemotherapy, radiotherapy or surgery. Oestrogen deprivation as a result of POI has serious implications for female health. In particular, bone mineral density, cardiovascular health, neurological systems and sexual health, may be impacted. The challenge posed by this important condition is the absence of standardized diagnostic criteria and management guidelines within our clinical practice. There are no local data about the causes and prevalence of POI in South Africa or adherence to international recommendations for management.

The aim of this study was to review the women who have presented to our gynaecological endocrine service with POI and to assess their diagnosis and presentation. Based on this information we plan to adjust, where necessary, our current protocol of investigations.

Methods

The study was conducted at the Gynaecological Endocrine clinic, at Groote Schuur Hospital (GSH), South Africa over a period of 11 months (June 2016 to May 2017). It was a retrospective folder review of women diagnosed with POI from 1983 to date. Ethics approval was granted by the Human Research Ethics Committee of the Faculty of Health Sciences of UCT [HREC REF:315/2016] and further permission to access patient records was given by the Hospital committee.

A total of 442 patients with the diagnosis of POI were identified using the card index system in our Gynaecological Endocrine Clinic. Clinical folders and microfilms were reviewed and information transferred to a template. The data were then entered using a Microsoft Excel spreadsheet and analysed. A total of 303 patients aged less than 40 presenting with primary

or secondary amenorrhoea/oligo-menorrhoea of at least 6 months' duration with serum FSH concentrations of >25mIU/mL on at least two occasions were evaluated. Comparison between groups was done using the t-test with a p-value of less than 0.05 being considered significant.

Results

A total of 369 patients with POI were identified in our clinic and we were able to review 303 of these clinical records (66 missing). Patients were aged 12-40 years at the initial visit. Serum levels of FSH, LH and oestradiol were similar in patients with primary and secondary amenorrhoea. Chromosomal abnormalities were more likely in the 38 patients with primary amenorrhoea (57.6%) than in those with secondary amenorrhoea (23.6%). Of 237 patients who presented with secondary amenorrhoea, more complained of symptoms of oestrogen deficiency (78.2%) and had been pregnant before diagnosis (53.2%) than those with primary amenorrhoea ($p<0.001$).

Immune disturbances were present in 4.6% patients, mostly in women with secondary amenorrhoea. The most common karyotype in the 38 patients with primary amenorrhoea was 45X0 (n=18). Of the patients with primary amenorrhoea 4 had gonadal dysgenesis. After completing investigations, the cause was not identified in 36.3% (n=110) of the patients, followed by genetic causes 20.8% (n=63), chemo/RT 9.6% (n=29), iatrogenic 5.0% (n=15) and autoimmune causes 4.6% (n=14). Investigations were incomplete in 22.8%(n=72) of the women due to failure to continue follow-up.

Conclusion

It is important to offer a comprehensive assessment to women with POI to establish the cause and institute appropriate treatment. Counselling on long term management and fertility options is essential. Many women do not complete investigations after receiving the initial diagnosis and greater awareness of POI needs to be developed, along with increased education of women planning fertility later in life, particularly if they are at risk of POI. Women with POI have unique needs that require special attention and our clinical services need to address these.

Chapter 1 Introduction

Premature ovarian insufficiency is a clinical syndrome defined by loss of ovarian activity before the age of 40. It is characterized by menstrual disturbance (amenorrhoea or oligomenorrhoea) with raised gonadotrophin and low oestradiol concentrations (1).

As a woman approaches menopause, there is a gradual cessation of ovarian function, leading to oestrogen deficiency and a reduction in fertility. This is associated with a rise in gonadotrophin levels. The mean age of this natural phenomenon is 52 years. When this occurs below the age of 40, it is defined as premature ovarian insufficiency (POI) (2,3).

Traditionally clinicians have characterized POI by the following triad:

1. Amenorrhoea for at least 6 months
2. Sex steroid deficiency
3. Two recordings of serum concentrations of follicle stimulating hormone (FSH) of more than 40mIU/L at least one month apart (3).

More recently, the European Society of Human Reproduction and Embryology guidelines (ESHRE) recommend the following criteria:

1. Oligo/amenorrhoea for at least 4 months, and
2. An elevated FSH level >25mIU/L on two occasions >4 weeks apart (4)

1.1 Literature review

Premature ovarian insufficiency is a primary ovarian defect characterized sometimes by absent menarche (primary amenorrhoea) or premature depletion of ovarian follicles before the age of 40 years (secondary amenorrhoea). When this happens between the ages of 40-45, it is termed early menopause as opposed to the permanent cessation of menses which occurs at the age of 52 years (5).

As defined by the World Health Organization(WHO), premature ovarian insufficiency is the failure of the ovary to function adequately in a woman younger than 40 years, both in its role as an endocrine organ and as a reproductive organ. In women 40 years or older, the expected physiologic decline of ovarian function that takes place with ageing is termed peri-menopause or the menopausal transition (4).

POI is a sub-class of ovarian dysfunction where the cause is within the ovary. In many women, the cause is unknown. The potential diagnosis includes genetic defects, chemotherapy, radiotherapy, surgery, autoimmune conditions and infections. In many women the cause will be idiopathic. Most commonly patients will present with absence of regular cycles, and the diagnosis is confirmed by raised serum FSH and low oestradiol levels (6).

In the largest clinical series (n=358) recorded by Bidet et al (2011) in Paris, France who looked at the resumption of ovarian function and pregnancies in 358 patients with POI, a definitive etiology was identified in only 44(12.3%) of these women (7). They were followed over a 13-year period (1997 to 2010) and the prevalence and predictive factors for spontaneous resumption of ovarian function were determined. When POI has been diagnosed, ovarian function is different from natural menopause. There is evidence that subsequent follicle development and ovulation do occur. In this study, some women with POI and amenorrhoea for 3-6 months, had follicle growth and 16-49% ovulated. The variability in the rates of follicle development and ovulation may be explained by the differences in duration of amenorrhoea. The rate of ovulation appears to decrease with a longer duration of amenorrhoea (7).

There is often an underlying genetic basis that may cause the disorder to occur in some women, or may simply predispose the individual to develop the disorder during their lifetime. Given that POI does occur within family settings, it is clear that some genetic defects may cause, or more likely are associated with the development of POI(8).

The disorder has considerable impact on reproductive health when it arises at a young age, and long term deprivation of oestrogen has serious implications for female health and quality of life in general including bone density, cardiovascular and neurological systems,

psychological wellbeing and sexual health. Health, is defined by WHO as a complete state of physical, mental, spiritual, and social well-being (9). The diagnosis of POI can have an impact on all these aspects of health.

1.2 Incidence

POI can be primary (spontaneous), or secondary (induced by radiation, chemotherapy, or surgery). Even though there have been many studies on the mean age at menopause, the incidence of POI is not well established. According to Coulam and Adamson who followed a cohort of 1858 women in Rochester, Minnesota, USA, POI was estimated to affect approximately 1% of women under the age of 40 years, 0.15% of women under 30 years and 0.01% of women under the age of 20 years of age (10,11).

It is, however, likely that the incidence of POI will continue to rise as the cure rates of cancers in childhood and among young women continue to improve, as per the report from the childhood cancer survivor study by Sklar et al (2006) (12).

In a cross- sectional study of women aged 40-55 years conducted by Luborsky et al at several sites in the USA to determine the general prevalence of POI by ethnic group, POI was reported by 1.1% (126/11 652) women which are similar results to what Coulam and Adamson found in their cohort of 1858 women of Rochester, Minnesota, USA (11,13).

1.3 Nomenclature

Various terms have been suggested to define deviation from healthy ovarian function, including premature menopause, premature ovarian failure and primary ovarian insufficiency. Many recent reviews have discussed the appropriate term for the condition Alternative terms have been proposed for this condition such a premature ovarian failure, premature ovarian dysfunction and more recently primary ovarian insufficiency (14). The term premature ovarian failure suggests that the state of ovarian failure is irreversible, which is not strictly true. In addition, there are negative connotations of ‘failure’ for a woman who has just received a traumatic diagnosis which impacts future fertility. Some authors have

used the term premature ovarian dysfunction (POD), in an attempt to reflect the possible reversible nature of this condition and to avoid the idea of failure (15).

Premature menopause is also an inappropriate term to describe POI. At one time, it was believed that FSH levels in the menopausal range were definitive evidence of ovarian follicle depletion, equivalent to irreversible and permanent cessation of ovarian function resulting in the use of the term premature menopause (16).

Authors more recently have utilized the term premature ovarian insufficiency (POI). This term communicates a sense that the pathophysiology represents a continuum. In addition, the term may be more acceptable to patients in that it reflects some measure of hope with regards to spontaneous recovery from POI (3, 4).

Based on evidence that POI has a long and variable clinical course that is not encompassed by its label, it has been proposed by Nelson et al that physicians return to the more accurate term used by Fuller Albright: Premature ovarian insufficiency (POI) (6).

This term is not only more accurate, but also informative for patients who may not experience the end of ovarian function at the time of diagnosis. The term POI may be preceded by descriptors that identify the severity of ovarian dysfunction.

The current recommended term premature ovarian insufficiency (POI) will be used in our study.

1.4 Pathogenesis

Women are endowed with their maximum number of follicles at approximately 20 weeks gestation. The normal process of atresia then commences independent of ovulation. At birth, the number of oocytes are decreased from 6-7 million to 700,000. At puberty, only 400,000 follicles remain and continued atresia along with monthly ovulation leave few follicles remaining at menopause (17). As a result of this constant oocyte atresia, early menopause

may occur in any woman who begins life with a decreased number of follicles, or experiences accelerated follicle apoptosis or destruction.

As documented by Goswami et al, ovarian insufficiency can be caused by a primary disorder in the ovary or it can occur as a result of secondary causes. Ovarian insufficiency is considered primary if the ovary fails to function normally in response to appropriate gonadotrophin stimulation provided by the hypothalamus and pituitary gland. Ovarian insufficiency is considered secondary if the hypothalamus and pituitary fail to produce appropriate gonadotrophin stimulation (18).

POI is, in reality, a continuum of disorders as highlighted earlier. Ovarian insufficiency may be divided into 4 clinical states and, these states are not necessarily permanent. Patients may move from one state to another in an unpredictable manner. In some cases, normal ovarian function may return for a brief period of time (19).

1. Occult POI: Presents as unexplained infertility in a patient with a normal basal FSH level. These patients have an inexplicable failure to respond adequately to FSH therapy during attempts at inducing ovulation.

2. Biochemical POI: Presents as unexplained infertility in patients with an elevated basal serum FSH. These patients also fail to respond adequately to FSH therapy during ovulation induction.

3. Overt POI: Previously referred to as POF or PM. This clinical state is characterized by elevated basal FSH levels in association with disordered menstrual cycles.

4. Premature ovarian insufficiency: The extreme state of complete primordial germ depletion. This is an irreversible state characterized by the presence of amenorrhoea, permanent infertility and gonadotrophins elevated to menopausal levels.

Table 1.1 Summary of clinical findings in women with POI (19)

Clinical state	FSH level	Fecundity	Menses
Normal ovarian function	Normal	Normal	Regular
Occult POI	Normal	Reduced	Regular or irregular
Biochemical POI	Elevated	Reduced	Irregular
Overt POI	Elevated	Reduced	Irregular or absent
POI	Elevated	Zero	Absent

1.5 Etiology of POI

The causes of POI are heterogeneous and largely unknown. In most cases, the cause of POI remains undetermined(idiopathic), but acquired forms such as those occurring following treatments for neoplastic diseases and autoimmune disease account for many cases.

Some of the different causes can be defined as follows:

- Iatrogenic (surgery, chemotherapy, radiation);
- Autoimmune, including polyglandular autoimmune syndrome;
- Infections (e.g. herpes zoster, cytomegalovirus);
- Chromosome X defects:
    - Turner syndrome
    - Fragile X syndrome
- Monogenic defects
    - Congenital disorders of glycosylation
    - Galactosaemia (recessive)
    - Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (female-limited, dominant)
- Isolated defects:
    - FSH receptor mutations (recessive)
    - LH receptor mutations (recessive)

- FOXL2 (transcription factor involved in BPES) mutations (female-limited defect, dominant)
- Bone morphogenic protein 15 (BMP15) mutation (female limited defect, heterozygous mutation)

Based on the above, most investigators find it convenient to subdivide patients with POI into two distinct categories: patients with follicle depletion and patients with follicle dysfunction. These are discussed in detail in the next section(16).

Follicle depletion

Follicle depletion is a major pathogenic mechanism for the development of POI. The presence of normal numbers of follicles in the ovaries (approximately 300,000-400,000 at the beginning of puberty) is crucial for normal ovarian function. Full maturation of one dominant follicle is dependent on the simultaneous development of a support cohort of non-dominant follicles. These, although destined to undergo atresia, play an important role in the fine – tuning of the hypothalamic-pituitary-ovarian axis by secreting regulatory hormones such as estradiol, inhibins, activins and androgens (20,21).

Thus an initial deficiency in primordial follicles or an accelerated rate of follicular atresia which results in premature depletion of the initial follicle endowment may result in POI. A disruption in any step of germ cell formation and migration, oogonia proliferation and meiosis results in a deficient initial follicle number.

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Causes of follicle depletion

1. Accelerated follicle atresia/destruction

X chromosome monosomy/aneuploidy or mosaicism (as observed in Turner syndrome or some cases with 47XXX karyotype) are associated with follicle atresia. Approximately 1 in 2500 females are born with Turner syndrome, and manifest characteristic features including congenital lymphedema, short stature and ovarian failure. The presentation and genetic background can be variable. Approximately 50% of patients have a 45X0 karyotype; however,

5-15% carry the long arm of the X chromosome along with the normal X chromosome 46X, i(Xq); and the rest have chromosomal mosaicism, for example 45X/46XX (16).

Women with Turner syndrome have a normal complement of oocytes until the third month of fetal life, after which apoptosis is accelerated, resulting in oocyte depletion by the first decade of life. As a consequence, only 10% of women with Turner syndrome achieve menarche. In contrast, patients with Turner syndrome due to mosaicism (45X/47XXX) are more likely to menstruate (40%) and can do so for several years before developing overt POI. The rate of atresia, although accelerated, is relatively slower and these women present with secondary amenorrhoea (22).

Another X chromosome disorder in which POI is well described is the Fragile X syndrome premutation, characterized by medical, psychiatric and cognitive features, where the prevalence of POI is 13-26% and requires targeted investigations (23).

2. Galactosaemia

Galactosaemia is a genetic disorder resulting from mutations in galactose 1-phosphate uridyltransferase (GALT), the enzyme that converts galactose to glucose. Patients with classic homozygous mutations have no GALT enzyme activity and therefore build up galactose metabolites in multiple cell types. These products cause cell damage through mechanisms that are not entirely clear. Clinically, homozygous patients present with mental retardation, liver failure and renal insufficiency. Women develop POI that can occur early enough to result in primary amenorrhoea (24).

Maintenance of a galactose free diet eliminates or reverses most clinical symptoms in galactosaemia. In a questionnaire based study about nutritional habits, 295 women aged 38-49 years, showed that a galactose consumption which exceeded 6g per day resulted in significantly higher FSH levels than women who consumed less galactose-containing nutrients (24).

3. Surgery

Women who have undergone a unilateral oophorectomy have higher FSH levels and a poorer response to ovarian stimulation than women who have retained both ovaries. They also have an earlier menopause. Thus by logical extension, POI may occur if significant ovarian tissue is lost through surgery (25).

4. Chemo- and radiation therapy

Iatrogenic POI is principally a result of cancer related treatment, which can be in the form of surgical procedures such as hysterectomy and oophorectomy or treatment with radiotherapy or chemotherapy.

The likelihood of developing POI depends on the woman's age and dosages of radiation or chemotherapeutic agents used. The latter impair maturation of follicles and decrease the primordial pool. The pre-puberty ovary is relatively resistant to this form of gonadotoxicity (26).

In a multicentre Childhood Cancer Survivor Study by Charles et al at the National Cancer Institute in the USA where the incidence of and risk factors for POI in survivors who were older than 18 years was assessed, 6.3% developed acute ovarian failure defined as never menstruating or developing amenorrhoea within 5 years of the cancer diagnosis and 8% developed POI. In the same study the most significant risk factor for acute ovarian failure was radiation therapy. However published data of the risk and frequency of POI in these patients is limited (12).

Radiation therapy damages the dividing granulosa cells, while the oocyte is relatively resistant. In some cases, the ovaries can be moved out of the radiation window, although scattered radiation may still cause ovarian damage and the uterus may also be affected. During chemotherapy, the oocyte is damaged in the resting state(27).

5. Inflammation

A further cause of POI is autoimmune oophoritis, in which inflammatory cells invade the theca cells of pre-antral and antral follicles, impairing their function. Primordial follicles however are spared. Thus, in this situation POI is predominantly a case of follicle dysfunction. Autoimmune oophoritis may occur alone or in addition to other autoimmune endocrine disorders(28).

6. Smoking

Smoking is associated with a decrease of age at menopause by 5-6 years. A large epidemiological study by Alida et al looked at factors associated with age at onset of menopause in a cohort of 3,650 UK women. The study also demonstrated a dose dependent impact of smoking on fertility which is seen from a half pack per day (29).

Follicle dysfunction

Some patients with POI have numerous ovarian follicles with seemingly normal oocytes which fail to develop and ovulate in the presence of elevated gonadotrophins. Most of these patients have idiopathic disease, but in some cases, a specific cause can be found.

Causes of follicular dysfunction

1. Specific gene defects

Abnormalities of the FOXL2 gene (Forkhead transcription factor gene) cause blepharophimosis, ptosis and epicanthus inversus syndrome. This is a rare congenital dysplasia of the eyelids, which is usually inherited as autosomal dominant. In these patients, the ovaries initially contain many follicles that do not grow (resistant ovaries), and later ovarian follicle depletion occurs (16).

2. FSH and LH receptor gene abnormalities

The role of gonadotrophins, particularly FSH, in initiating growth and development of early postnatal follicles, is not completely understood. Studies of a mouse model with knocked out

FSH receptor (FORKO mice), have shed some light on the role of FSH in development and maintenance of the primordial follicle pool. In this animal study, Balla et al observed that in adult Follitropin Receptor Knockout(FORKO) female mice (infertile and oestrogen deficient mice), FSH receptor ablation resulted in a decreased number of growing ovarian follicles, reduced in diameter, with ovaries that were 45% smaller and the mice also had 40-50% smaller uteri and vaginas. They concluded that the FSH receptor system might be a major contributor to the growth of the ovarian follicle (30).

3. Signaling defects

These are related to FSH and LH receptor abnormalities (30).

4. Autoimmunity

The immune system may play a role in some causes of POI. The real prevalence of autoimmune POI is unknown. The presence of circulating antibodies to the ovary in serum samples from some women with POI, may suggest an immune mechanism in the pathogenesis. (31).

Numerous case reports exist of histological findings consistent with autoimmune oophoritis. The ovaries are of normal size or are enlarged. The patients with autoimmune oophoritis may also have circulating anti-adrenal and/or steroid cell antibodies with unclear functional significance. These may be regarded as markers of autoimmune attack against steroid hormone-producing cells (both in the ovaries and adrenal glands) (32).

POI is also frequently associated with auto-immune disorders, including hypothyroidism, insulin dependent diabetes mellitus and antibodies including parietal cell antibodies and acetylcholinesterase antibodies identified in myasthenia gravis. In 1985, Michael et al conducted a study at the Ottawa Civic Hospital in Canada (1979-1982). The purpose was to assess the relationship of POI to autoimmune disease. Thirty-three patients with the diagnosis of chromosomally competent POI were reviewed. The study demonstrated that 39% had an associated autoimmune disorder (33).

5. Infection

A true cause and effect relationship between POI and infection has not been established. Keay et al from the University of Bristol in their commentary, reported of a series of case reports which recorded organisms as diverse as salmonella, schistosomiasis and mumps virus affecting the ovary (34).

1.6 Clinical presentation of premature ovarian insufficiency

For most women, there are no obvious signs or symptoms that precede the cessation of menses. Most commonly, menstrual disturbance is the presenting complaint, although symptoms of oestrogen deficiency such as vasomotor symptoms, mood disturbances, change in sleep cycle, dyspareunia caused by atrophic vaginitis, among others, may be present (2).

The most severe forms of POI present with absent pubertal development and primary amenorrhoea. The clinical picture is characterized by absent menarche and delay in pubertal development resulting in absent sexual maturation and reduced growth velocity. Pubertal delay is defined as absence of breast and pubic hair development and menarche at the age of 13(35).

As stated previously, in addition to amenorrhoea and vasomotor symptoms, POI can also occur as a result of ovarian surgery, oophorectomy or exposure to viral or environmental toxic agents such as smoking, chemotherapy and radiotherapy for cancer treatment.

1.7 Differential diagnosis of POI

There are several conditions that have a similar presentation and need to be excluded as causes of amenorrhoea. These conditions are listed below and parameters useful for the exclusion of each of the conditions are also illustrated.

- Pregnancy: High chorionic gonadotrophin levels.
- Hypothalamic (Kallman syndrome): low oestrogen and progesterone.
- Pituitary disease (pituitary tumours): high prolactin and low/normal gonadotrophin levels, alterations in imaging of brain/sella tursica region.
- Hypothalamic amenorrhoea (induced by stress, intensive exercise, anorexia, weight loss, fasting, severe disease): low/normal gonadotrophin levels, low oestradiol.
- PCOS: Typical ovarian ultrasound, normal gonadotrophins, raised serum androgen levels, hirsutism and menstrual dysfunction.
- Other endocrine disorders (hyper and hypothyroidism, Cushing syndrome): normal gonadotrophin levels.
- Enzymatic defects of steroidogenesis (21-hydroxylase deficiency) (CAH): alterations on adrenal ultrasound, high androgen and 17α-hydroxyprogesterone (17α-OHP) levels.

1.8 Ovarian insufficiency assessment

Initially, a detailed menstrual history is important as most commonly, dysfunctional menstruation is the presenting complaint although symptoms of oestrogen deficiency, such as vasomotor symptoms, mood disturbances and dyspareunia may be present.

A family history of POI can be recorded in 14-31% of cases and a definite familial disorder can be identified in some of these. Perrault syndrome, FSH receptor mutations and Fragile X permutations are relatively more common among women with a family history of POI.

The clinician should enquire about the family history when the diagnosis of POI is suspected. The enquiry should include genetic causes such as mental retardation and autism spectrum disorders in children and tremor, Parkinson syndrome in adults to screen for potential Fragile X permutation carriers (23).

The possible familial relationship of POI has been documented from small epidemiological studies. There is evidence that suggests a familial component in some cases as reported by

Vegetti et al in Italy, who investigated the incidence and inheritance pattern of familial POI after they analysed 71 cases (8).

A physical examination, including height, weight and body mass index is essential and the recognition of any dysmorphic features suggestive of chromosomal or genetic cause of POI is important. The skin should be examined for hirsutism, acne, striae, acanthosis nigricans and vitiligo. Pubertal development must be staged.

1.9 Diagnostic criteria for Primary Ovarian Insufficiency as recommended by the 2016 ESHRE guidelines (4)

1. Oligo/amenorrhoea for at least 4 months, and
2. Elevated FSH level >25mIU/L on two occasions >4 weeks apart.

Three groups of tests should be performed when POI is suspected, these are:

1. Tests to establish the diagnosis
2. Tests to clarify the etiology
3. Screening tests for other diseases that are known to have a higher prevalence among women with POI.

1.10 Management of women with POI

The management of women with POI should address the prevention and treatment of oestrogen deficiency symptoms (endocrine health), genetic health, specific fertility management (reproductive health options), education, counselling and psychological support (emotional health).

1.11 POI in South Africa

There are very little data on POI in Sub-Saharan Africa or South Africa. The prevalence of POI is not known. Because of improvement in the treatment of childhood and adult malignancies and radical surgery due to endometriosis and malignancy, this may have increased in recent years.

The majority of studies and reports on this condition have been in the industrialized world. We have examined the presenting features and clinical status of patients with POI attending the Gynaecology Endocrine Clinic at Groote Schuur Hospital, Cape Town, South Africa. Based on this information, we hope to gain insight on the presentation and management of these patients and we plan to adjust, where necessary, our current protocol of investigations.

Chapter 2 Methods

2.1 Aim

The aim of the study was to review women who have been diagnosed with POI in our service and to assess their presentation and diagnosis.

2.2 Objectives

2.2.1 Primary Objective

The main objective was to review women who have been diagnosed with POI at Groote Schuur Hospital (GSH) in the Gynaecological Endocrine clinic and to assess their diagnosis.

2.2.2 Secondary objectives

To assess the presentation and investigations in women presenting with POI.

2.3 Settings

The study was conducted at Groote Schuur Hospital (GSH) within the Gynaecological Endocrine clinic (GEC). GSH receives referrals from the Western Cape and other provinces in South Africa, including the Eastern Cape. It has a busy but well-structured Gynaecological Endocrine clinic.

2.4 Methods

2.4.1 Study Design

This was a retrospective clinical audit utilizing case records of women who presented to our service and were diagnosed with POI. We reviewed the clinic folders which were identified mostly through a card index which we keep for all women attending the GEC.

2.4.2 Sample size and Eligibility

Patients were identified in the card index of attendees at the Gynecological Endocrine Clinic from 1985 to 2017 and their folders were then reviewed.

Eligible study participants were patients with a confirmed diagnosis of premature ovarian insufficiency, per the new ESHRE diagnostic criteria.

2.4.3 Data collection

A template was designed for the collection of the data and this is attached (Appendix 1). Data were collected over a period of 6 months (December 2016-June 2017). The template was based on how these patients are managed in the GEC clinic from history taking, physical examination to investigations and management.

In the GEC, all the patients have a complete history and a physical examination performed as well as a number of routine laboratory tests as illustrated in Appendix 2 with reference ranges included. These laboratory tests may be adjusted according to the patient presentation and include chromosomal analysis. Normal ranges may change when new assays are introduced and this has been taken into account when assessing results over time.

Data collected included:

- Age at first visit
- Age at diagnosis of POI (often earlier than first visit)
- Total number of visits
- Educational background
- Presenting complaints
- Previous consultations elsewhere
- Obstetric and contraceptive history
- Past and present medical, gynaecological and surgical history
- Family and social history
- Physical examination
- Diagnostic laboratory blood and imaging tests to establish diagnosis

Medical management and fertility treatment if required

Psychological wellbeing

Final diagnosis

The data template was completed by the principal investigator. The collection of data from the first 15 folders was piloted and adjustments made where some of the questions were unclear and the data template was then modified with the permission of HREC (Appendix 3 and 4).

Data were entered twice into a data base on Microsoft Excel 2012 spreadsheet, initially by the principal investigator, then again by a data entry clerk within the Department of Obstetrics and Gynaecology at GSH. The two separate sets of data were cross compared using the software package Excel compare, discrepancies were checked and corrected.

All the completed data forms were securely stored in the Reproductive Medicine Unit.

2.4.4 Ethics Approval

The study was initially approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF 315/2016) (Appendix 3).

All data were treated confidentially. Patients were identified by their folder numbers and data were only accessible to the principal investigator, the research nursing sisters and the supervisor. The records were anonymized for statistical analysis.

Permission to access the patient folders for review was provided by Groote Schuur Hospital Chief Operational Officer Dr Bernadette Eick (Appendix 5).

The study was undertaken in accordance with the guidelines of the Helsinki Declaration on Human Experimentation 2013(36).

2.4.5 Statistical Analysis

This was a descriptive study. Data analysis was done using STATA version 12.

Demographic data are presented in a descriptive manner using summary graphs that were generated in Excel. For numerical data, such as age, parity and gravidity data are described with means, modes, and medians. In the analysis of categorical data, tables were used.

Comparison between groups was done using the t-test with 2-sided p-value of less than 0.05 being considered significant. The t-test is one type of inferential statistics. It is used to determine whether there is a significant difference between the means of two groups. With inferential statistics, we assume the dependent variable fits a normal distribution. When we assume a normal distribution exists, we can identify the probability of a particular outcome. We specify the level of probability (alpha level, level of significance, p) we are willing to accept before we collect data (<0.05 being a common value that is used).

2.4.6 Dissemination of information

This review will be submitted as part fulfillment for the degree MMed (O&G) within the University of Cape Town. The results were presented as a poster presentation at the 38th Congress of the South African Society of Obstetricians and Gynaecologists (SASOG) in March 2018.

It is planned to submit a manuscript for publication in a peer-reviewed journal.

The results will be available to our clinical services.

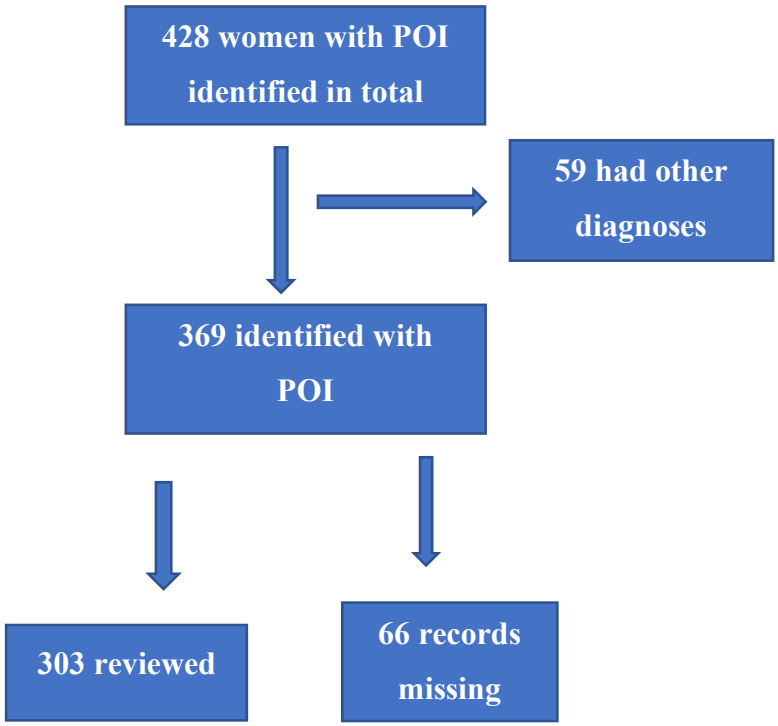
Chapter 3 Results

Initially 428 women were identified from the card index of patients attending the GEC. We excluded 59 who were found to have other final diagnoses, which included: early menopause, polycystic ovary syndrome, Sheehan's syndrome and Mullerian agenesis. Finally, 369 women were identified with the diagnosis of POI.

Subsequently we were able to review 303(82.1%), of these clinical records as 66 (17.9%) were not available. These patient records included 21 microfilms and 282 patient clinical records.

Figure 3.1 summarizes the patient population reviewed.

Figure 3.1 Review of patient population



Total women with POI = 369

66 missing records = 17.9%

303 reviewed = 82.1%

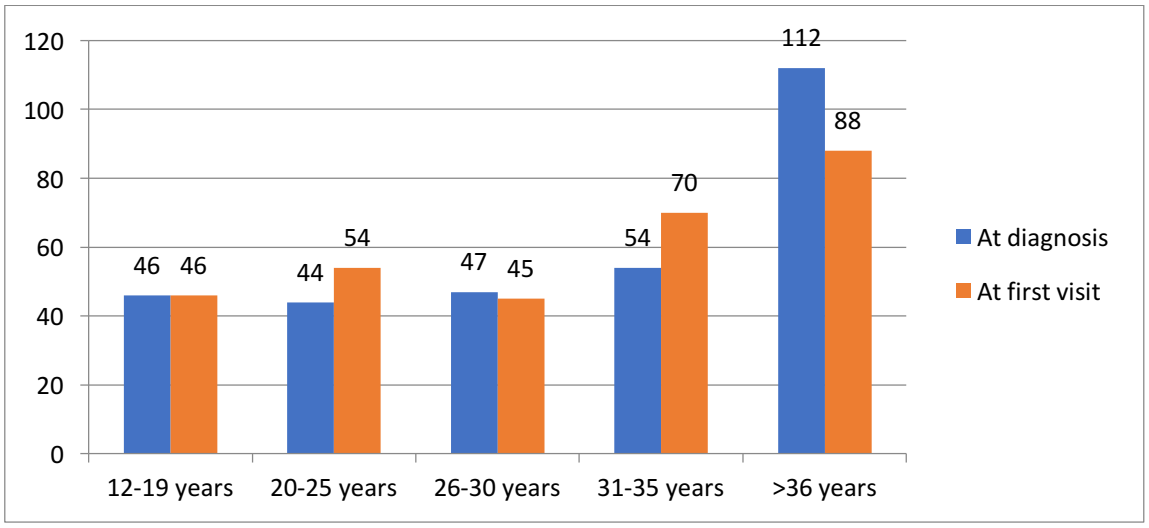
3.1 Demographic characteristics of patients

3.1.1 Age

The mean age at diagnosis was 30.2 years (SD = 5.801) (Range 12- 39 years).

The age distribution among study patients at first visit and age at diagnosis is illustrated in Figure 3.2.

Figure 3.2 Age at diagnosis and age at first visit



3.1.2 Number of visits

The first patient reviewed presented in 1985. The minimum number of visits per patient was one visit and the maximum number of visits for one patient was 58 visits (n=1) (range 1-58).

3.1.3 Referrals

Most referrals were in-house gynaecology referrals n=120 (39.6%), with 27 patients being referred from other disciplines which included: radiation oncology, haematology and medical endocrinology. This is illustrated in table 3.1

Table 3.1 Referral source

Discipline	Number of referrals
Primary care	9.2% (n=28)
Secondary care	36.3% (n=110)
Gynae GSH	39.6% (n=120)
Other disciplines	8.9% (n=27)
Unknown	5.9% (n=18)
Total	**100% (n= 303)**

Table 3.1 displays from where our patient population was referred with 39.6% (n=120)/303 being gynaecological in-house referrals.

3.2 Presenting symptoms

A summary of the common presenting symptoms and their frequency is illustrated in Table 3.2

Table 3.2 Presenting symptoms

Symptom	Yes (%)	No (%)	Don't know (%)	Unrecorded (%)
Hot flushes	122 (40.3)	144 (47.5)	0 (0)	37 (12.2)
Night sweats	73 (24.1)	175 (57.8)	0 (0)	55 (18.2)
Reduced libido	16 (5.3)	178 (58.8)	9 (3.0)	100 (33.0)
Vaginal dryness	22 (7.3)	171 (56.4)	9 (3.0)	101 (33.3)
Infertility	129 (42.6)	108 (35.6)	6 (2.0)	60 (19.8)
Dyspareunia	25 (8.3)	165 (54.5)	10 (3.3)	103 (34.0)
Primary amenorrhoea	66 (21.8)	237 (78.2)	0 (0)	0 (0)
Oligo/secondary amenorrhoea	237 (78.2)	66 (21.8)	0 (0)	0 (0)

Oligo/secondary amenorrhoea was the most common presenting complaint (78.22%) followed by infertility 42.57% and hot flushes 40.26%. In our audit, we found that in 122(40.3%)women, hot flushes, a symptom of oestrogen deficiency were reported. Only 8 (6.6%) of the patients with primary amenorrhoea complained of this symptom, whereas 114 (93.4%) of those with secondary amenorrhoea did so ($p<0.001$).

3.3 Clinical features of women with POI

Table 3.3 summarizes the clinical features of patients with POI. Most patients (n=220) had consulted about their complaints elsewhere before referral to the gynae-endocrine clinic at GSH. At the time of presentation, 80 patients had one or more known pre-existing medical illnesses. One woman had Down's syndrome and 18 had Turner syndrome already diagnosed before being referred to the GEC.

Table 3.3 Clinical features of women with POI

No. of patients	303
Mean age at diagnosis	30.2 (range 12-39)
Mean age of menarche in women with secondary amenorrhoea	13.9
Previous consultations elsewhere	220 (72.6%)
Current or previous sexual activity	207 (68.3%)
Pre-existing medical illness	80 (26.4%)
Pre-existing growth disorder	18 (5.9%)
Previous surgical history	99 (32.7%)
Investigations complete	161 (53.1%)

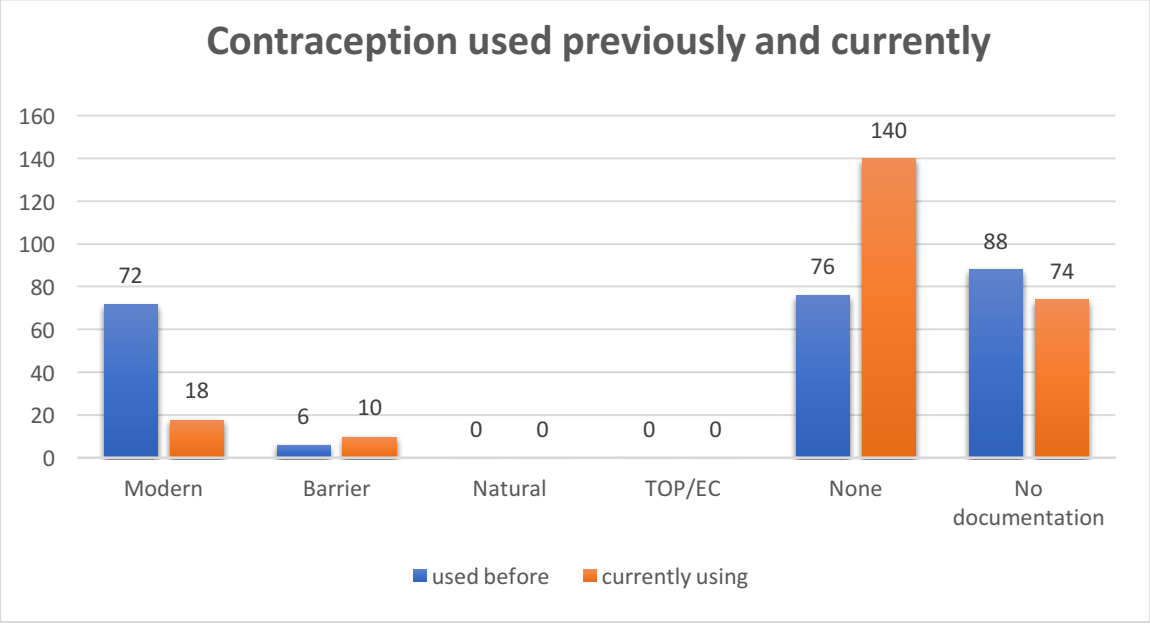
Previous surgical history was varied and included hysterectomy, oophorectomy which might have contributed to POI, hemicolectomy, breast lumpectomy, myomectomy, kidney transplant, unilateral mastectomy, tonsillectomy, exploratory laparotomy, diagnostic laparascopy and burr-holes following head trauma.

While 168 women were either currently or previously sexually active, this was not recorded for 74 women, and 61 reported to have never been sexually active.

3.4 Contraceptive history

For analysis, contraceptive or interceptive methods were divided into four categories: modern, barrier, natural and termination of pregnancy (TOP)/Emergency contraception. Figure 3.3 illustrates the distribution of contraception previously and currently used.

Figure 3.3 Contraception used



At the time of presentation 140 of the women (67.6%) who were sexually active were not using any form of contraception. A total of 72 women had previously used modern contraception which included the following: the combined oral contraceptive pill (COC), the mini pill, depo-medroxyprogesterone acetate(DMPA), the IUD, Mirena (IUS), long term

implants and female and male sterilization. Barrier methods used, were the male and female condoms and the cap/diaphragm.

None of the patients reported having used or were using either a natural method nor termination of pregnancy(TOP) or emergency contraception(EC) as a form of fertility regulation, 61 women had never been sexually active.

3.5 Obstetric history

Of the 303 women reviewed, 58.4% (n=177) were nulligravida at the time of presentation to the GEC. Of all the women who had been pregnant (126/303), twenty did not have a viable pregnancy. The obstetric history of patients we reviewed is presented in Table 3.4

Table 3.4 Obstetric history

Gravidity	Number	Percentage (%)
0	177	58.4
1	58	19.1
2	37	12.2
3	21	6.9
4	3	1.0
5	4	1.3
7	1	0.3
9	2	0.7
Total	303	
Parity	Number	Percentage (%)
0	197	65.0
1	58	19.1
2	29	9.6
3	14	4.6
4	3	1.0
6	2	0.7
Total	303	
Number of surviving children	Number	Percentage (%)
0	198	65.4
1	58	19.1
2	31	10.2
3	11	3.6
4	3	1.0
6	2	0.7
Total	303	

3.6 Malignancies and chemo/radiation therapy

A total of 39 women (12.9%) had previous malignancies, and 25 (8.3%) of these women had received chemo-radiation therapy (see table 3.5)

Table 3.5 Malignancies and chemo/radiation therapy in women with POI

Malignancy	Chemo/RT	No chemo/RT
Haematological (n=19)	13	6
Gynaecological (n=8)	2	6
Non-gynaecological (n=12)	10	2
Total	**25**	**14**

Non-gynaecological cancers included a wide variety of conditions including Kaposi sarcoma, osteosarcoma, adenocarcinoma of the colon, colorectal carcinoma, myxofibrosarcoma, myxoid-lymphosarcoma, and rhabdomyosarcoma.

3.7 Marital status

In our study, 103 women (34%) were married, 86 were single and not in a relationship, 43 were single in a stable relationship but not cohabiting, with 22 in a stable relationship and cohabiting. The marital status was not recorded for 38 women. In addition, 10 women were either separated or divorced, and there was one widow.

3.8 Smoking history

At the time of presentation, 57 women were smokers of whom 9 were heavy smokers, smoking more than half pack per day. A total of 191 were non-smokers and smoking history was not recorded for 55 women.

3.9 Clinical findings

3.9.1 Comparison of women with POI presenting with primary or secondary amenorrhoea

As summarized in Table 3.6 some differences and similarities existed between those patients presenting with primary and those with secondary amenorrhoea.

Table 3.6 Comparison of women with POI presenting with primary or secondary amenorrhoea

	Primary amenorrhoea[a]	Secondary amenorrhoea[a]	Significance[b]
Stigmata of Turner syndrome	34 (72.3%)	13 (27.7%)	<0.001
Chromosomal abnormalities	38 (67.9%)	18 (32.1%)	<0.001
Osteopenia	23 (29.5%)	55 (70.5%)	<0.001
Osteoporosis	13 (31.7%)	28 (68.3%)	<0.001
Pregnancies before diagnosis	0 (0%)	126 (53.2%)	<0.001
Delayed puberty	53 (81.5%)	12 (18.5%)	<0.001

[a] Values in parentheses are percentages.

[b] Results calculated using X^2 test.

3.9.2 Physical stigmata for Turner syndrome

Turner syndrome was diagnosed in 49 women who presented with POI. On observation and physical examination, 47/49 patients had stigmata of Turner syndrome, and these included short stature, webbed neck, shield-like chest, wide carrying angle of elbows, and Madelung deformity.

The 45 patients with a height <150cm were more likely to have an abnormal karyotype compared to those whose height was >150cm (t-test <0.005).

3.9.3 Chromosomal abnormalities

A total of 114 chromosome analyses were done, of which 58 (50.9%) were normal 46XX karyotype and 56 (49.1%) had abnormal karyotypes. Most of the older patients who had secondary amenorrhoea did not have karyotyping.

Chromosomal abnormalities in women tested were more common in those with primary amenorrhoea (67.9%, n=38) than in those with secondary amenorrhoea (32.1%, n=18) ($p<0.001$) and moreover these two groups of women also had different types of chromosomal abnormalities (Table 3.7). The common stigmata for Turner syndrome documented in the patient records was short stature (n=42) and was mostly in women with primary amenorrhoea. The shortest woman had a height of 1.22m with a karyotype of 45X0.

As can be seen from Table 3.7 most of the women were diagnosed with Turner syndrome and had classic monosomy, 45X0 as a common karyotype. Four of the women with primary amenorrhoea were found to have XY chromosomes and they all had a gonadectomy. One of the women with secondary amenorrhoea had 47XXX karyotype.

Table 3.7 Chromosomal abnormalities

Primary amenorrhoea karyotype (n=38)	Number	Secondary amenorrhoea Karyotype (n=18)	Number
Turner Syndrome		Turner Syndrome	
Isochromosome 46i(xq)	1	45X/46X+mar(3)	1
45X0/46X, isoX	3	45X0	4
45X/46X,isoX	1	46X0	2
45X0	15	45,X,i(x)(q10)[23]/45X[7]	1
47X0	1	45X[50%]/46,X,tr[50%]	1
46X0	4		
45X0/46X,r(X)	1		
46,X,tmar[20]/45,X[10]	1		
Turner mosaic		Turner mosaic	
45X(60%), 46XY(26%)47XYY	1	46X,isox(X)	1
45,X[9]/46,X,tmar[11]	1	45X[34]/46Xtmar45X0	1
45X[23]/46X, isoX[17]	1	46XX/47XXX	1
46,X,t(x;17)(q13;p112)	1	47XXX[2]/46,XX[28]	1
45X0(80%)/46XX(20%)	1	46XX/47XXX(ExtraXchromosome)(22%)	1
Turner syndrome variant			
45,X[13]/46,X.r(X)(??)[7]	1		
45X/46Xdel(X)(q22.1-pter)	1		
46,X,del(X)(P10)	1		
Gonadal dysgenesis			
46XY FEM	4		
		Deletions	
		46X,del(X)(q21.2)	1
		46Xq28microdel	1
		46,X,del,(X)(p11.22-pter)	1

Figure 3.7 indicates the distribution of genetic causes in patients with POI

3.10 Investigations

3.10.1 Basal hormonal levels

Mean serum levels of gonadotrophins and steroids were similar in patients with primary and secondary amenorrhoea. As expected LH and FSH concentrations were markedly increased with values as high as 200mIU/ml.

Basal concentrations of the two FSH measurements varied greatly between patients.
Levels of basal prolactin were similar in women with primary and secondary amenorrhoea and were within the normal range of 4.79-23.3ng/ml for most patients. Prolactin levels varied considerably.

Table 3.8 Gonadotrophins and sex steroids

	FSH1 (mIU/ml)	FSH2 (mIU/ml)	LH (mIU/ml)	E2 (pmol/L)	TESTOSTERONE (nmol/L)	SHBG (nmol/L)	FAI
Minimum	18.8	14.1	2.7	5	0.1	5.2	0.005
Maximum	200	200	200	950	3.0	149.3	3.50

FSH1 and FSH2 refers to the diagnostic tests for POI.

Androgens were measured for in 105 women. As mentioned in sub-section *2.4.3* normal reference ranges have varied over the years. Most of our patients had low androgen levels.

The testosterone level of one woman was documented as 11.1nmol/L. This result has been excluded as this was probably an incorrect entry, unfortunately the GEC notes could not be traced to check on the high result.

3.10.2 Immune disturbances

Immune disturbances were identified in 14 of the patients (4.62%), and always in women with secondary amenorrhoea. The immune disturbances occurred in patients who presented with secondary amenorrhoea and who also had normal karyotypes. Some of these women were asymptomatic, and the abnormalities were detected only during investigation.

Seven women had signs associated with autoimmune disease. These included Lichen planus, eczema of the ears, dry skin, exophthalmos and lichen sclerosis.
Seven women had documented thyroid disease, mostly hypothyroidism, making this the most common associated endocrine disorder, with one patient documented as having had a thyroidectomy. A total of 8 women had anti-thyroid peroxidase titers >35IU/ml (normal <35 IU/ml), and most of these women were asymptomatic.

Lupus Nephritis was reported in 5 women. One patient was documented as having had a kidney transplant; 2 patients had eczema; 2 patients had Systemic Lupus Erythematosus; one had Lichen Planus; one had ulcerative colitis and one had scleroderma.

3.10.3 Bone density

Two hundred of the 303 women who had the bone mineral density of the lumbar spine and hip checked, had reduced bone density assessed by the T-score as shown in Table 3.9 below. Of the women who had reduced BMD, 83 presented with secondary amenorrhoea compared to the 36 women with primary amenorrhoea ($p< 0.001$).

One woman previously had a pathological fracture and was started on bisphosphonate therapy prior to presentation.

Table 3.9 Bone mineral density (BMD) among women with POI

	Normal BMD (T-score -1.0 and above)	Osteopenia (T- score -1.0 and -2.5)	Osteoporosis (T-score -2.5 and lower)	Total
Primary amenorrhoea	10 (21.7%)	23 (50.0%)	13 (28.3%)	46 (100.00)
Secondary amenorrhoea	71 (46.1%)	55 (35.7%)	28 (18.2%)	154 (100.00)
Total	81 (40.5%)	78 (39.0%)	41 (20.5%)	200 (10.00)

Figure 3.9 illustrates that 41/303 (20.5%) women already had osteoporosis by the time they first presented.

3.10.4 Imaging

Eighty women had a pelvic ultrasound scan to assess the uterus and ovaries. The minimum volume of the ovaries was 0.005ml and the maximum volume was 35ml with a mean ovary volume of 4.1ml.

CT-brain was performed on 5 patients of which 3 had normal findings, 2 had micro adenomas with one of them also having hydrocephalus.

3.10.5 Additional investigations in women with Turner syndrome

Echocardiogram was performed in 39 women who had Turner syndrome and pathology was detected in 3 of them. The pathologies detected included bicuspid regurgitation, coarctation of the aorta and a ventricular septal defect (VSD).

Audiograms were performed in 21 of these women and pathology found in 11 women. Bilateral sensorineural hearing loss was found in 3 of these women and the other 8 had mild bilateral hearing loss.

3.11 Treatment

Medical management was with hormone replacement therapy, mostly with the COC (n=260), or oral estrogen. There were no patients who were prescribed the combined patch, estrogen patch or androgens. A total of 43 patients had no medical treatment. A total of 147 patients were prescribed additional supplements including calcium and vitamin D but 146 patients were not given medical supplements. Bisphosphonate treatment was offered to one patient who already had a fragility fracture at presentation.

In terms of fertility treatment, 69 patients were offered In-vitro fertilization (IVF) and oocyte donation, only 7 pursued this option. For a further 81 patients, IVF + oocyte donation was discussed but the patients never returned for further consultation . Sixty-two patients could not afford the cost of IVF, and two further patients opted for adoption or fostering. This is outlined in Table 3.10

Table 3.10 Treatment received by women with POI

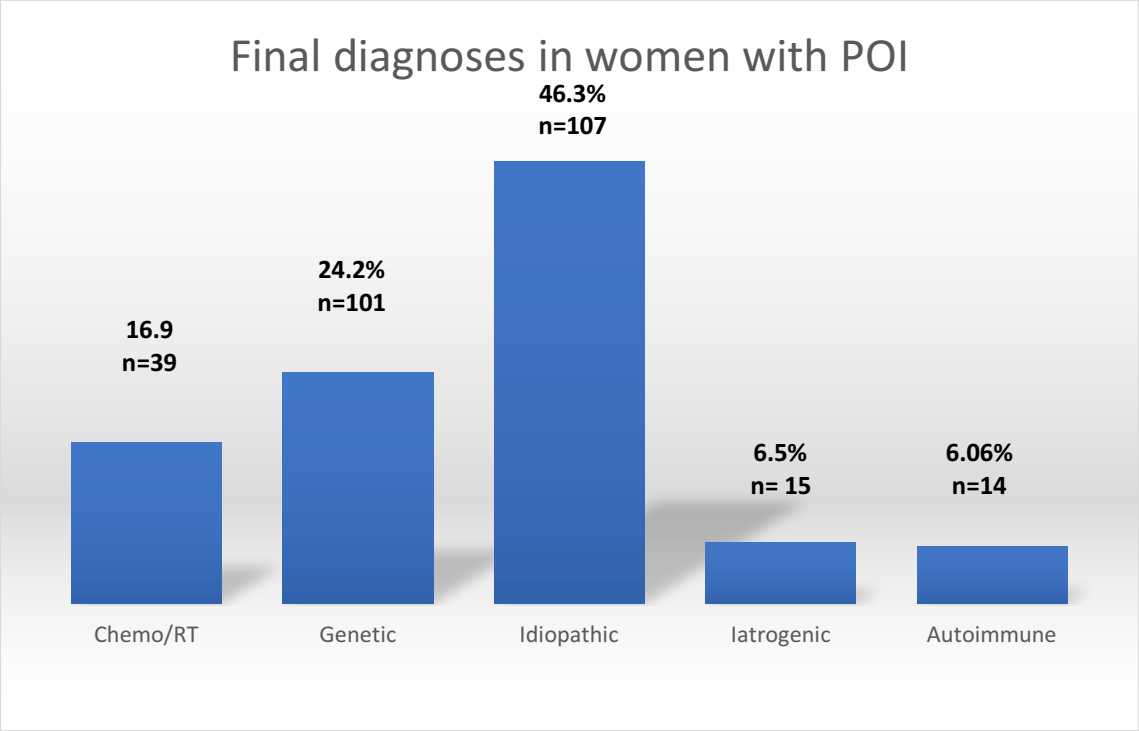
Treatment modality	Number
Gonadectomy	8
Induction of puberty	40
Hormone replacement therapy	260
Calcium and vitamin D	147
Bisphosphonates	1
IVF	7
Adoption/Fostering	2

3.12 Psychological wellbeing

Referral for additional supportive counselling was documented in 53 patient records, of whom seven declined this counselling. In 243 patient records, counselling was not recorded. Forty-nine women gave a history of ever needing support for depression and low mood, and were offered counselling.

3.13 Final diagnosis in women with POI

Figure 3.5 Final diagnoses



A final diagnosis in women with POI was made in 231 women and is illustrated in Figure 3.5. None of the women were found to have POI secondary to infection and unfortunately investigations were incomplete in 23.8% women (n=72) and who never returned for follow up.

Chapter 4 Discussion

Premature ovarian insufficiency(POI) is a clinical syndrome defined by loss of ovarian activity before the age of 40. POI is characterized by menstrual disturbance (amenorrhoea or oligomenorrhoea) with raised gonadotrophin and low oestradiol levels. To the best of our knowledge, this is the first study in South Africa which audits patients with POI.

This was a retrospective folder review of patients diagnosed with POI the in Gynaecological Endocrine clinic at Groote Schuur Hospital in South Africa. The aim of the study was to obtain information about the women who have presented to our service with premature ovarian insufficiency and to review their diagnoses and investigations. Ultimately we hope to develop a protocol for investigations and management for POI which will be used in our service and will be based on findings of this audit. Going forward we hope to contribute to the International database on POI.

The population sample of 303 women out of 428 potential subjects was a reasonable representation of women with POI in our clinical services. Of those not analyzed the reasons included 59 women who were incorrectly labelled as POI in the card index when in fact they had other diagnoses such as gonadal agenesis, ambiguous genitalia or early menopause. A second problem encountered was that many cards were not accurately completed in the clinic and information was accessed by extensive review of all folders. Those patients who had stopped attending the Gynaecological Endocrine Clinic (GEC) for a variety of reasons, had their folders either micro-filmed or had their GEC clinical folders stored in records. Sixty-six of these booklets were missing and could not be retrieved by the record clerks, partly due to poor record keeping and partly as some of these clinic notes were filed by other clinical departments where these women were also being managed for other medical conditions.

To make a diagnosis of ovarian failure, traditionally clinicians have used the age of 40 as the cut-off, and amenorrhoea for 3-6 months combined with FSH levels above 40iu/L. The American College of Obstetrician and Gynaecologists (ACOG) in 2014 defined the exact criteria for their diagnosis of POI. More recently ESHRE (2016) presented a clear guideline.

According to ESHRE, the patient population should comprise women younger than 40 years, with oligo/amenorrhoea of at least 4 months and elevated FSH level >25IU/L on two occasions >4 weeks apart. In our study, the inclusion criteria of patients followed ESHRE guidelines, bearing in mind that many clinicians were not aware of this new guideline and thus were still using the traditional criteria and excluding some patients who deserved to have the diagnosis confirmed as the threshold for FSH level is now lower (4,37).

The primary objective of our study was to review women who have been diagnosed with POI at the Gynaecological Endocrine clinic at Groote Schuur Hospital (GSH) and to assess their diagnosis. The secondary objective was to assess the presentation and investigations in these women. We hoped to recommend future management strategies in this group of women based on our findings. Our results are in keeping with other studies which have shown that although several causes for POI have been described in a significant number of women, the etiologic factor for most patients still remains unknown, even after thorough investigation. In our study, we found that for 36.3% of patients, the cause for POI was unknown. No studies could be identified that had looked at the percentages of women with POI in different local South African population.(1,6).

In this audit, most patients (39.6%) who were seen in the Gynaecological Endocrine Clinic were in-house gynaecology referrals, followed by secondary care referrals (36.3%), primary care referrals (9.2%), other disciplines (8.9%) and in 5.9% of patients, the referral was not documented. The in-house referrals had baseline investigations done to evaluate amenorrhoea, so that by the time they were seen in the GEC, a diagnosis was possible, thus reducing the time to confirm the diagnosis for the patient. In the folders which were reviewed, the baseline evaluation included a pregnancy test, and if negative, measurement of serum FSH, prolactin and thyroid function. This shows that most of the clinicians' knowledge is good as the suspicion that the diagnosis might be POI in young women with irregular or absent menses is considered.

Symptoms due to oestrogen deficiency

The various conditions may influence the different symptoms in these women. It has been well documented in the literature that women with primary amenorrhoea less often show symptoms of night sweats, depressive mood, flushing and vaginal dryness. On the other hand, women with iatrogenic POI, due to surgery or cancer therapy are most often symptomatic (38).

James et al in their multi-center study in the USA, Canada and Australia, done to evaluate the efficacy and safety of a testosterone patch in surgically menopausal women with hypoactive sexual desire disorder found that up to 50% of post-oophorectomized women reported a decrease in sexual desire. They hypothesized that this decrease may be associated with lower androgen levels due to the oophorectomy. Women who have undergone oophorectomy typically experience a big and sudden drop of circulating androgen hormone levels. Direct studies of POI and vasomotor symptoms are limited(39).

In our audit, the basal oestradiol levels in women with primary and secondary amenorrhoea did not differ. The low frequency of symptoms of estrogen deficiency in the women with primary amenorrhoea, despite their tendency to have lower oestrogen concentrations, supports anecdotal conclusions that such symptoms are due to oestrogen withdrawal. This conclusion is also supported by the observation that some women with primary amenorrhoea complaining of symptoms had been treated with exogenous estrogen in the past. Women with primary amenorrhoea may not have vasomotor symptoms but these women may develop such symptoms if they have withdrawal of HRT after receiving this HRT for a period of time. However, in this audit, we did not document whether patients had been on exogenous oestrogen therapy prior to presentation at the GEC. (40).

The South African Menopause Society (SAMS), presented a revised consensus position statement on Menopausal Hormone Therapy in 2014 and recommended that HT or oral contraception be used at least until the natural age of menopause. Subsequently,

recommendations for hormone therapy can be followed up utilizing recommendations for women with natural menopause (41).

After the various clinical issues have been addressed, patients can review aspects of family planning in a comprehensive manner. Although many women with POI desire a pregnancy, it is not the case with everyone. It is important to make it clear to patients, that spontaneous remission of POI sometimes occurs, and that unexpected pregnancy occurs in 5-10% of women with this condition (7).

Some couples desire parenthood but are uncomfortable with adoption or reproductive technology as a solution. They are content to define their family as a couple and accept the real, albeit low chance that they will conceive without medical intervention. For couples ready to pursue parenthood actively, the options are adoption, foster parenthood, egg donation and embryo donation (42,43).

Contraception and Pregnancy

Most of these patients had presented with infertility and were seeking assistance with this clinical problem. Some had stopped using contraception as they did not see the need to continue because of amenorrhoea which they related to not being able to fall pregnant.

It is important to inform women with POI that there is a small chance of spontaneous pregnancy and thus they should use contraception if they wish to avoid pregnancy. A more recent analysis by Bidet et al who followed a cohort of 358 women in Paris, France, revealed that 25% of women showed evidence of ovarian function, and that pregnancy occurred in 4.8%. Thus, it is important for the clinician to inform these patients that even though there was evidence of anovulation and high circulating gonadotrophins, there was a small possibility of intermittent ovulation (7).

Embryo cryopreservation, ovarian tissue cryopreservation and oocyte cryopreservation hold promise in cases where ovarian failure is foreseeable as in women undergoing cancer treatments

Recent data indicate that facilitating pregnancy in women with Turner syndrome is particularly problematic as these women are at increased risk of aortic rupture during pregnancy. Even if the echocardiogram fails to show any dilatation of the root of the aorta, they may still be at risk of rupture during pregnancy because of the abnormal structure of the wall of the aorta. These data indicate that adoption or surrogacy should be encouraged (44).

Malignancies and chemo/radiation therapy

In our study, thirty-nine women were treated for malignancies which were both gynaecological and non-gynaecological related. The treatment varied from surgery alone to surgery to chemo/radiation or both. Gynae-related malignancies were identified in 8 women, of whom only 2 women had received chemo/radiation therapy. The malignancies were endometrioid adenocarcinoma Grade 1 and borderline serous ovarian carcinoma. Section 3.7 in the results section, outlines the other non-gynaecological malignancies.

The loss of ovarian function during or shortly after completion of cancer therapy is known to occur in those who are exposed to high dose pelvic irradiation and/or intensive alkylating agent based regimens that are used for cytoreduction. However, in our study, we did not have documentation of what chemo-therapeutic agents were used.

Chemaitilly et al in their retrospective cohort, multi-center study on acute ovarian failure in the Childhood Cancer Survivor Study in the United States found that radiotherapy to the ovaries was the most significant risk factor for POI, a finding that is consistent with previous reports before them (45). The previous data indicate that radiation doses affect the ovaries in a dose -dependent fashion.

For those women who require HRT after malignancy, especially following treatment for hormone sensitive tumours, liaison with the oncology team is essential. Each case should be treated individually. In those with hormone sensitive tumours, it is advised that there should be a delay of at least 6-12 months before initiating therapy to reduce the risk of recurrence.

Published data pertaining to the risk and frequency of POI in cancer survivors are limited. Advances in treating cancer have resulted in markedly improved survival rates. It is estimated that 70% of children and adolescents diagnosed with cancer can expect to be long term survivors and because of this, we will see more young women with POI. These data will assist clinicians in counselling patients and their families at the time of diagnosis and before cancer treatment is initiated(45).

Basal Hormone Levels

Serum levels of gonadotrophins were similar in our patients with primary and secondary amenorrhoea.

La Marca et al in their study compared women with POI secondary to steroidogenic autoimmunity to those with idiopathic POI. They observed that those women with autoimmune POI had significantly lower levels of FSH (n=26, range 26-64mIU/ml) compared to those with idiopathic POI (range 61-166mIU/ml) (p=0.001). We did not demonstrate this relationship in our study.

The presence of raised FSH levels can no longer be termed indicative of irreversible ovarian failure. A growing body of literature documents evidence of follicular activity and even pregnancies in women with confirmed POI (5,7,18,32).

Immune Disturbances

These potentially serious associated disorders mandate that patients with POI be evaluated thoroughly. In this study, some of the measurements of autoimmune screening tests were not conducted at Groote Schuur laboratory, but would have been conducted at other laboratories depending on the site at which each patient was initially evaluated. We therefore had differences in the reference ranges for the laboratory values, making analysis difficult. In some patients who were seen in the GEC as early as 1985 when the clinic started, and did not have their laboratory results in their patient notes. It was difficult to trace these

results as the new automated laboratory system of the National Health Laboratory Services (NHLS) only goes back for five years.

In our study, we found that immune- associated abnormalities were detected more often in the women who presented with secondary amenorrhoea than in those with primary amenorrhoea.

The relationship between POI and autoimmune disorders is well documented. About 50% of patients with POI have ovarian antibodies, but the clinical relevance of these is undefined because of their high prevalence (31%) in patients with normal ovarian reserve as demonstrated by Novasad et al at the National Institute of Child Health and Human Development, Bethesda, Maryland USA. In their study on ovarian antibodies, they concluded that ovarian antibodies as detected by the indirect immunofluorescence methods have poor specificity, and are rarely useful. The specificity of any ovarian antibody test should be established before it is used clinically.

In our clinic thyroid antibodies are used as a surrogate to check for the presence of associated autoimmune disease as we cannot access the other antibody screens(46).

Chromosomal Abnormalities

Chromosomal abnormalities and failure to undergo complete secondary sexual development were more common in the women with primary amenorrhoea. Not all the patients who had a diagnosis of premature ovarian insufficiency had a chromosomal analysis done. Most patients with primary amenorrhoea had karyotyping (56 out of 66 patients) as compared to those who presented with secondary amenorrhoea (58 out of 237 patients). Patients with secondary amenorrhoea were tested based on age at presentation and physician judgement.

The frequency of karyotype abnormalities in patients with primary amenorrhoea (38/66, 57.6%) was significantly higher than in those with secondary amenorrhoea (18/237, 7.6%, $p<0.001$), The most common abnormality was a structural abnormality involving the X chromosome. Similar to our findings, Jiao et al also reported similar findings in their

Cytogenetic analysis of 531 Chinese women with POI. In their study, they detected chromosomal abnormalities in 12.1% of their study population, a figure that was lower compared to our findings of 20.8%. The difference might be due to the fact that in their study they had excluded those patients with typical Turner stigmata and those with conditions known to induce POI (chemo-or radio-therapy, surgery or autoimmune disease) (47).

One patient who was Turner mosaic warrants mention. This 33-year-old woman had presented with secondary infertility following a miscarriage at 20 weeks, five years before presentation to the GEC and being diagnosed with POI. She had undergone puberty that was normal in both timing and development, with menarche at 12 years of age. Her weight was 69kg and her height 1.45m, and her calculated BMI 32.9. There were no significant clinical signs. The pelvic examination was normal and pregnancy test was negative. FSH levels were in the menopausal range. Her chromosomal analysis revealed that she was a Turner mosaic.

Turner syndrome is a common genetic disorder (50 per 100,000 live-born females) that has been classically associated with a 45XO karyotype. Several X-chromosomal abnormalities have been identified in these patients, many which involve mosaicism. These patients have variable but predictable phenotypic findings such as short stature, webbed neck, a shield-like chest with wide spaced nipples, a wide carrying angle of the elbows, and deafness, among other presentations. They are at risk for development of endocrine, autoimmune and structural abnormalities. An estimated 1-2% of all patients may become pregnant (48).

Many affected women do not progress through puberty unless they receive hormone therapy, and most are unable to conceive. Some literature has documented that a small percentage of women with Turner syndrome retain normal ovarian function through young adulthood, however only a few studies have evaluated the rate of spontaneous pregnancies.

We identified four women who had a Y chromosome. When the Y chromosome is identified on karyotyping, usually a prophylactic gonadectomy is recommended because of the increased risk of gonadoblastoma, a benign neoplasm of the dysgenetic gonad. The incidence of gonadoblastoma is estimated at 12% in Turner syndrome patients carrying the Y chromosome.

Women with POI who are found to have an abnormal karyotype and those with Fragile X mental retardation (FMR) permutation will require genetic counselling. These diagnoses may affect the patients' health but they may also have important health implications for her relatives and potential offspring. A trained genetic counsellor can effectively convey this information to patients and family members. Ongoing counselling and access to family follow-up is essential(49).

Bone Mineral Density

In our study, 200 of the 300 women who had a BMD assessment had reduced bone density indicating either osteopenia or osteoporosis. Reduced BMD has been reported in several studies investigating women with POI due to different etiologies, compared to reference populations. Our audit is in keeping with findings by Vaishali et al (2009) in Bethesda, Maryland USA, where they conducted a cross sectional study of 442 women with POI and assessed bone density and associated risk factors for reduced bone density(50). In their study, women with POI had a lower BMD compared to controls even after adjusting for race, age and BMI in a regression model. Because of the increased risk of osteoporosis in women with POI, it is important to optimize factors that maintain bone health, such as adequate calcium intake, vitamin D levels and weight bearing exercises.

A total of 8% of bone mass is lost in the first year after menopause and thereafter between 1% and 2% is lost annually. The vertebral bone is more sensitive to oestrogen deficiency and vertebral fractures tend to occur in a younger age group (50-60 years) than fractures in the femoral neck (≥70 years). In our study, women who had more than one year delay in diagnosis of POI after the onset of menstrual irregularities were more likely to have a lower BMD compared to those women who were diagnosed earlier after the onset of their symptoms. We noted that from the onset of menstrual irregularity to the time they were seen at our institution, the duration of taking HRT varied due to a variety of reasons which included: non-adherence, delay in diagnosis and confusion regarding the use of HRT. This shows that delay in HRT, is significantly related to lower bone density in women with oestrogen insufficiency. Thus clinicians, parents and adolescents should view the menstrual

cycle as an important sign for initiating therapy as it will minimize the delay in diagnosis and maximize the chance of achieving a healthy peak bone mass.

Psychological wellbeing

The diagnosis of POI carries a significant emotional impact. Infertility as a consequence of POI can affect a woman's self-esteem, identity, sexuality and relationships with others. In most cases of infertility due to other causes, the problem becomes apparent after many failed attempts at conception, and, usually, such couples identify the infertility problem themselves. In contrast, the inability to conceive as a result of POI is usually diagnosed suddenly and unexpectedly (6,7).

In our study, in a minority of patients (n=53), additional supportive counselling was documented, where the patients were referred to see a social worker. In 49 of these women, a history was given of depression, low mood and needing support. In some patient records women expressed distress after speaking to their clinician and receiving the diagnosis of POI, but also in the long term with regard to infertility.

An association between POI and psychological distress has been reported, and it has been suggested that psychological care should be included in its management. Studies specifically conducted on women with POI, demonstrate lower self-esteem and increased shyness and social anxiety. In one observational study by Groff et al at the National Institute of Health (NIH) in Maryland, USA, where the study aimed at learning more about the emotional processes associated with POI via focus groups and structured interviews, they found that most of the women, (81%) stated that their diagnosis made them feel angry, 76% felt depressed, 74% felt generally less healthy and 63% reported an altered self-image (51).

Schmidt and colleagues in their study at the NIH in Maryland, USA showed that women with POI had a higher frequency of depression and greater lifetime risk of depression. Thus, these women should be evaluated for underlying depression and offered expert care (52).

Given the emotional impact of POI, physicians confirming the diagnosis need to take the time necessary to explain the disorder and to answer questions. Communicating the diagnosis in a caring fashion and assessing the patient's emotional support system can help women to cope with this diagnosis. If appropriate, and with the patient's consent, informing partners and other family members regarding the challenges these women face can help patients create an environment of trust, warmth and support at home. Physicians may possibly recommend other avenues of emotional support such as social workers or other mental health professionals.

Existing literature shows an association between POI and psychological distress, and suggestions have been made that psychological care should be included in its management. From our study, the fact that 76 patients did not return for follow-up, may reflect the psychological distress that the diagnosis causes and the fact that these women do not want to continue with further investigations (4,51-53).

Based on the findings of our study, the following investigations for women who present with POI in our clinical environment are recommended:

1. FSH and LH (Done 4 weeks apart).
2. Prolactin.
3. Thyroid function tests (TSH, FT3, FT4).
4. Cortisol (0800).
5. Insulin and glucose. Glucose tolerance test (GTT) if indicated.
6. Karyotyping, unless a specific cause of POI has been identified such as chemo-radiation.
7. Pelvic ultrasound.
8. Bone age in women with absent or delayed secondary sexual development.
9. Bone mineral density in women with prolonged oestrogen deficiency.
10. Turner syndrome: echocardiogram and audiogram.
11. Collagen screen and auto-immune screening.
12. Counselling at initial visit to prevent defaulting and offer psychological support.

There were some limitations in our review. Missing records were a challenge to the data collection. In addition, missing documentation by clinicians in the patient folders reduced input in data collection. There is no standard format on how clinicians document their findings. It was also observed that some clinicians were not clear about the diagnostic criteria for POI. It is possible that we may therefore have missed some patients who had the diagnosis.
The findings from this study are limited by its retrospective format. Our findings point to the need for more research in this area, and perhaps prospective studies to compare support strategies in these women is important.

The strength of our audit was in the large number of women with POI who had adequate clinical records which we could review and the fact that this is the first comprehensive review of POI patients in South Africa from a dedicated clinic. It is recommended that new research areas need to be identified and prospective follow up patients with POI to assess the metabolic impact and longer term psychological and social implications as a result of premature ovarian insufficiency should be expanded.

Conclusions

In our study we found that our study population had 5 main etiologies which resulted in POI. These were idiopathic, iatrogenic, genetic, chemo/RT and autoimmune causes which is in concordance with other studies. Many women will not have an identifiable cause after all investigations have been completed and some may not complete follow-up investigations after receiving the initial diagnosis.

From the data we have accessed, we conclude that women with POI who present with primary and secondary amenorrhoea form separate populations with clinical characteristics which differ somewhat.

Improvement in the treatment of childhood malignancies and chronic illnesses such as Lupus Nephritis, gynaecological surgical practice and lifestyle may contribute to an increase in the prevalence of POI because of longer survival rates.

It is important for all health professionals to be well versed in diagnosis, management options and fertility counselling for women with POI. Ongoing audits of patients with premature ovarian insufficiency will be valuable for South Africa but require that an identification and monitoring system to be universally accepted.

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APPENDICES

Appendix 1. Data Template

RETROSPECTIVE REVIEW OF WOMEN DIAGNOSED WITH PREMATURE OVARIAN INSUFFICIENCY AT GROOTE SHUUR HOSPITAL

HREC REF: 315/2016

Folder number: ----------

First name: ----------

Surname: ----------

Case Number: ----------

Date of review: ----------

Demographic data

1. Date of first visit (DD/MM/YYYY)	
2. Date of last visit (most recent visit) (DD/MM/YY)	
3. Total number of visits	
4. Who referred the patient Primary care Secondary level referral Gynae in house tertiary referrals Other disciplines (specify)_____ Unknown	

5. Date of birth (DD/MM/YYYY)	
6. Age at first visit (In years and months)	
7. Age at diagnosis of POI (In years and months)	
8. What is the highest level of education attained? (1) No formal schooling (9) Grade 8 (2) Grade 1 (10) Grade 9 (3) Grade 2 (11) Grade 10 (4) Grade 3 (12) Grade 11 (5) Grade 4 (13) Grade 12 (6) Grade 5 (14) Tertiary (Complete) (7) Grade 6 (15) Tertiary (Incomplete) (8) Grade 7 (16) Unknown	

Presenting Complaints and Duration of problem

9. Hot flushes (1) Yes (2) No (3) Don't know (4) Not recorded	

10. Night sweats (1) Yes (2) No (3) Don't know (4) Not recorded	
11. Poor concentration (1) Yes (2) No (3) Don't know (4) Not recorded	
12. Reduced libido (1) Yes (2) No (3) Don't know (4) Not recorded	
13. Vaginal dryness (1) Yes (2) No (3) Don't know (4) Not recorded	
14. Infertility (1) Yes (2) No (3) Don't know (4) Not recorded	
15. Dyspareunia (1) Yes (2) No (3) Don't know (4) Not recorded	
16. Bone pain (1) Yes (2) No (3) Don't know	

(4) Not recorded	
17. Amenorrhoea/Oligomenorhoea (1) Yes (2) No (3) Don't know (4) Not recorded	
18. Delayed puberty (1) Yes (2) No (3) Don't know (4) Not recorded	
19. Breast tenderness (1) Yes (2) No (3) Don't know (4) Not recorded	
20. Depression/anxiety (1) Yes (2) No (3) Don't know (4) Not recorded	
21. Urinary symptoms (1) Yes (specify) _____ (2) No (3) Don't know (4) Not recorded	
22. Tiredness (1) Yes (2) No (3) Don't know (4) Not recorded	

23. Other (1) Yes (Specify) _____ (2) No	
24. Duration of problem at presentation	

Gynaecological History

25. Previous consultations elsewhere about current presenting complaint (1) Yes (2) No (3) Don't know (4) Not recorded	
26. Age at menarche (1) In years (2) No menarche (3) Don't know (4) Not recorded	
27. Last spontaneous menstrual period (1) In years and months (MM/YY) (2) N/A (3) Don't know (4) Not recorded	
28. Sexual activity (1) Sexually active (2) Previously (3) Never (4) Not recorded	

Contraceptive History (Spontaneous response)

Contraceptive Method	29. Used before	30. Currently using

(1) Pill (COC)		
(2) Mini pill (POP)		
(3) Injection (Depo)		
(4) Loop (IUCD)		
(5) IUS(Mirena)		
(6) Cap/Diaphragm		
(7) Male condom		
(8) Female condom		
(9) Long term implants		
(10) Rhythm method		
(11) Withdrawal method		
(12) Abstinence		
(13) Spermicides		
(14) Female sterilization		
(15) Male sterilization		
(16) Morning after pill		
(17) Termination/ abortion		
(18) None of the above		
(19) Other (Specify)		
(20) Never used		
(21) N/A		
(22) Not documented		
(23) Never sexually active		

Obstetric History

31. Ever been pregnant before? (1) Yes (2) No	
32. Gravidity	
33. Parity	
34. Miscarriage	
35. Ectopics	
36. TOP's	
37. Number of surviving children	

Medical History

38. Current or previous medical illnesses (Include all that apply) (1) Inflammatory Bowel disease (2) Vitiligo (3) DM (4) Lupus Erythematosus (5) Rheumatoid arthritis (6) Sclerodema (7) Psoriasis (8) Hashimoto's disease (9) Graves' disease (10) Thyroid disease (Other) Specify _____ (11) Sjogren's disease (12) Adrenal Insufficiency (13) Hypertension (14) Other (Specify) _____ (15) N/A	

39. Haematological disorders (Include all that apply) (1) Thrombophilia (2) Sickle cell disease (3) Thalassaemia (4) Other(specify)_____ (5) N/A	
40. Haematological malignancies (1) Acute Lymphoid Leukaemia (ALL) (2) Acute Myeloid Leukaemia (AML) (3) Hodgkin's Lymphoma (4) Non-Hodgkin's Lymphoma (NHL) (5) Multiple Myeloma (MM) (6) N/A (7) Other(specify)_____	
41. Benign gynaecological disorders (1) Endometriosis (2) Benign ovarian cysts (3) Fibroids (4) Other(specify)_____ (5) N/A	
42. Gynaecological malignancies (1) Cervical (2) Endometrial (3) Myometrial (4) Fallopian (5) Ovarian (6) Other(Specify) _____ (7) N/A	
43. Non-Gynaecological cancers (1) Breast (2) Colon (3) Rectal (4) Melanoma (5) Other (Specify)_____ (6) Thyroid carcinoma	

(7) N/A	
44. Growth disorders (1) Turner syndrome (2) Achondroplasia (3) Dwarfism (4) Acromegaly (5) Seckel syndrome (6) Silver-Russell syndrome (7) Other (Specify)_____ (8) Don't know (9) N/A	
45. Previous irradiation /chemotherapy (1) Yes (2) No (3) Don't know	
46. Previous surgery (1) Hysterectomy (2) Oophorectomy (3) Other(specify)_____ (4) Don't know	

Family History

47. Positive family history (Mark all that are relevant) (1) POI (2) Male mental retardation (3) Autoimmune disorders (Specify)_____ (4) Male infertility (5) Delayed puberty (6) Diabetes Mellitus (7) Hypertension (8) Malignancy (Specify)_____ (9) None known (10) Other (Specify)_____ (11) Not recorded	

Social History

48. Marital status (1) Single, not in a relationship (2) Single in a stable relationship but not cohabiting (3) Single and cohabiting (4) Married (5) Separated (6) Divorced (7) Widowed (8) Not recorded	
49. Employment status (1) Disability grant or Other Grant (2) Student (3) Employed (casual) (4) Employed (formal) (5) Self employed (6) Unemployed (7) Housewife (8) Not recorded	

50. Smoking history (1) Yes (2) No (3) Stopped more than 6 months ago (4) Stopped less than 6 months ago (5) Not recorded	
51. If YES, how many per day? (1) 1-5 (2) 5-10 (3) 10-15 (4) 15-20 (5) >20 (6) N/A (7) Not recorded	
52. Alcohol consumption (1) Yes (2) No (3) Don't know (4) Not recorded	
53. Recreational drugs (1) Yes (specify which) _____ (2) No (3) Stopped more than 6 months ago (4) Stopped less than 6 months ago (5) Don't know (6) Not recorded	

Observations and Examination

54. Height (m)	
55. Weight (kg)	
56. Body Mass Index (Enter actual calculated BMI)	

57. Physical stigmata of Turner syndrome/ other genetic syndrome (1) Yes (2) No (3) Not documented	
58. If YES, specify (1) Short stature (2) Webbed neck (3) Low set ears (4) Shield-like chest (5) Wide carrying angle of elbows (6) Deafness (7) Madelung deformity (8) Other (Specify) (9) N/A	
59. Signs of Thyroid disease (1) Yes (2) No (3) Not documented (4) Don't know	
60. If YES, specify (1) Goitre (2) Exophthalmos (3) Bradycardia or tachycardia (4) Cold and dry skin (5) Lid lag (6) Hair loss and skin changes (7) N/A	
61. Signs associated with presence of autoimmune disease (1) Yes (2) No (3) Not documented	

62. If YES, signs (1) Vitiligo (2) Premature graying of hair (3) Alopecia areata (4) Malar rash (5) Orthostatic hypotension (6) Exopthalmos (7) Tremor (8) Facial swelling (9) Joint swelling (10) Other (Specify)_____ (11) N/A	

Investigations

63. Diagnostic Laboratory blood tests	
Karyotype	
(2) FSH (i) Follicular phase: 3.1-7.9 mIU/mL (ii) Ovulation peak: 2.3- 18.5 mIU/mL (iii) Luteal phase: 1.4-5.5 mIU/mL (iv)Postmenopausal: 30.6- 106.3 mIU/mL	
(3) LH (i) Follicular phase: 1-18 mIU/mL (ii) Ovulation peak: 20-105 mIU/mL (iii) Luteal phase: 0.4-20.0 mIU/mL (iv) Postmenopausal: 15.0-62.0 mIU/mL	
Estradiol (E2) (i) Follicular phase: 46-607 pmol/L (ii) Ovulation peak: 315-1828 pmol/L (iii) Luteal phase: 161-774 pmol/L (iv)Postmenopausal:<18-201 pmol/L	
(5) Testosterone (0.3- 1.7 nmol/L)	

(6) SHBG (26.1- 110 nmol/L)	
(7) FAI (0.4-5.9)	
(8) 17 alpha-OHP (i) Follicular phase 1.0 - 4.5 nmol/L (ii) Luteal phase 0.8 - 8.8 nmol/L (iii) Oral contraception 0.6 - 5.8 nmol/L (iv) Postmenopausal 0.6 - 2.2 nmol/L	
(9) DHEAS (i) Prepubertal 0.5-1.7µmol/L (ii) Premenopausal 5.3-13.7 µmol /L (iii) Postmenopausal 0.2-9.4 µmol /L	
(10) TSH (0.27 - 4.2 mIU/L)	
(11) FT3 (3.1- 6.8 pmol/L)	
(12) FT4 (12- 22 pmol/L)	
(13) Prolactin (4.79- 23.3 ng/mL)	
(14) Fasting insulin (<25 mIU/L)	
(15) Fasting glucose (3.5- 5.5 mmol/L)	
(16) Glucose/Insulin ratio	
(17) Cortisol (i) 0700- 0900: 120- 620 nmol/L (ii) 1500- 1700: 85- 460 nmol/L	
(18) Pregnancy test (i) Positive (ii) Negative (iii) Not done	
(19) ACTH stimulation test (i) Normal function (ii) Hypo-adrenalism	

(iii) N/A	
(20) Lipogram: (i) HDL (> 1.2 mmol/L) (ii) LDL (<3 mmol/L) (iii) Total Cholesterol (<5 mmol/L) (iv) Triglycerides (<1.7 mmol/L)	
64. Autoimmune screen	
(1) Anti-thyroid antibodies (i) Thyroid peroxidase antibody(TPOAb) <35 IU/mL (ii) Thyroglobulin antibody (TgAb) <20 IU/mL (iii) Thyroid-stimulating immunoglobulin antibody (TSI) <140% (iv) Thyroid- stimulating hormone (TSH) receptor binding inhibitor immunoglobulin (TBII) < 1.75 IU/L	
(2) ANA (Antinuclear antibody test) (i) (>1= positive) (ii) (0.7-1= Equivocal) (iii) (<0.7= Normal)	
(3) CRP (C-Reactive protein) <3.0 mg/dL	
(4) Complement test (i) C3 (88- 206 mg/dL) (ii) C4 (13- 75 mg/dL)	

(5) Collagen screen: (i) Rheumatoid factor (<14.0 IU/mL) (ii) Anti-Double stranded DNA <10 IU/mL: Negative 10-15 IU/mL: Equivocal >15 IU/mL: Positive	
(6) Auto-immune tests (Titres) (i) Anti- mitochondrial antibodies (<1:40) (ii) Anti- smooth muscle antibodies(<1:40) (iii) Anti-parietal cell antibodies (<0= 20.0U)	
65.Imaging (Pelvic USS)	
(1) Left ovary (i) Volume (ii) Other	
(2) Right ovary (i) Volume (ii) Other	
(3) Uterus (i) Present (ii) Absent (iii) Blind ending vagina	
(4) Other findings (Specify)	
66. CT/MRI (1) Brain (2) Other organs (Specify) _____ (3) N/A	
67. Echocardiogram (1) Normal (2) Pathology (Specify) _____ (3) N/A	

68. BMD (1) Normal (2) Osteopenia (3) Osteoporosis (4) Not done	
69. Bone age	
(1) Bone age(YY/MM) (2) Chronological age at bone age(YY/MM) (3) N/A	
70. Audiogram (1) Normal (2) Hearing Impaired (3) Other(specify)_____ (4) N/A	

Treatment

71. Surgical management (mark all that apply) (1) Gonadectomy (2) Hysterectomy (3) Vaginoplasty (4) Not required	
72. Induction of puberty (1) Yes (2) No (3) N/A	

73. Medical management (Mark all that apply) (1) HRT (i) E2 + Progesterone (ii) Oral estrogen only (iii) COC (iv) Combined Patches (v) Estrogen patches only (vi) Androgens (2) No treatment	
74. Fertility treatment (Mark all that apply) (1) Ovulation induction (2) IVF + oocyte donation (3) Not required (4) Offered but never returned (5) Other (Specify)_____	
75. Other medical supplements (Mark all that apply) (1) Calcium (2) Vitamin D (3) Bisphosphonates (4) No supplements (5) Other (specify)_____ (6) N/A	

Psychological wellbeing

76. Additional supportive counselling (1) Yes (2) No (3) Don't know (4) Not recorded	
77. Ever needed support for depression/ low mood (1) Yes (2) No (3) Don't know (4) Not recorded	

78. Investigations complete (1) Yes (2) No (3) Don't know	
79. Return for follow up (1) Yes (2) No	
80. Final Diagnosis	

Appendix 2

Reference ranges of lab tests performed in our patient population

Laboratory test	Reference range
FSH	(i) Follicular phase: 3.1-7.9 mIU/mL (ii) Ovulation peak: 2.3- 18.5 mIU/mL (iii) Luteal phase: 1.4-5.5 mIU/mL (iv)Postmenopausal: 30.6- 106.3 mIU/mL
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Prolactin	4.79- 23.3 ng/mL
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Lipogram	(i) HDL (> 1.2 mmol/L) (ii) LDL (<3 mmol/L)

	(iii) Total Cholesterol (<5 mmol/L) (iv) Triglycerides (<1.7 mmol/L)
Anti-thyroid antibodies	(i) Thyroid peroxidase antibody(TPOAb) <35 IU/mL (ii) Thyroglobulin antibody (TgAb) <20 IU/mL (iii) Thyroid-stimulating immunoglobulin antibody (TSI) <140% (iv) Thyroid- stimulating hormone (TSH) receptor binding inhibitor immunoglobulin (TBII) < 1.75 IU/L
ANA (Antinuclear antibody test	(i) (>1= positive) (ii) (0.7-1= Equivocal) (iii) (<0.7= Normal)
CRP (C-Reactive protein)	<3.0 mg/dL
Complement test	(i) C3 (88- 206 mg/dL) (ii) C4 (13- 75 mg/dL)
Collagen screen	(i) Rheumatoid factor (<14.0 IU/mL) (ii) Anti-Double stranded DNA <10 IU/mL: Negative 10-15 IU/mL: Equivocal >15 IU/mL: Positive
Auto-immune tests (Titers)	(i) Anti- mitochondrial antibodies (<1:40) (ii) Anti- smooth muscle antibodies (<1:40) (iii) Anti-parietal cell antibodies (<0= 20.0U)

Appendix 3: Human Research Ethics Committee Approval

HREC REF: 315/2016

Prof Z van der Spuy
Obstetrics & Gynaecology
H-Floor
Old Main Building

Dear Prof van der Spuy

PROJECT TITLE: RETROSPECTIVE REVIEW OF PATIENTS DIAGNOSES WITH PREMATURE OVARIAN INSUFFICIENCY (MMed-candidate Dr N Chirwa)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 26 May 2016.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th June 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continue beyond the approval period. Please submit a Standard Closure form if the study is completed within th approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student Dr N Chirwa will be involved in this study.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the princip investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

Appendix 4. Human Research Ethics Committee consent for amendments

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

HUMAN RESEARCH ETHICS COMMITTEE
13 JAN 2017
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
✓ Approved	✓ Type of review: Expedited	O Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature Chairperson of the HREC		Date 14/1/2017

Note: All major amendments must include a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Comments from the HREC to the Principal Investigator:

Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	**12 January 2017**
HREC REF Number	**315/2016**
Protocol title	**RETROSPECTIVE FOLDER REVIEW OF PATIENTS DIAGNOSED WOTH PREMATURE OVARIAN INSUFFICIENCY AT GROOTE SCHUUR HOSPITAL**
Protocol number (if applicable)	
Principal Investigator	**Professor Zephne Van Der Spuy**
Department / Office Internal Mail Address	**Department of Obstetrics and Gynaecology**

1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	O Major	O ✓ Minor
1.2 Does this protocol receive US Federal funding?	O Yes	O ✓No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval?	O Yes	O ✓No

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval. This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.
When we originally submitted our protocol we anticipated reviewing 150 women with premature ovarian insufficiency. To date we have identified about 400 women in our clinic and we hope to review all these folders, if available.

3. Protocol status (tick ☐)

✓	Open to enrolment
○	No participants have been enrolled
○	Closed to enrolment (tick ☐) ○ Research-related activities are ongoing ○ Research-related activities are complete, long-term follow-up only ○ Research-related activities are complete, data analysis only

4. Proposed changes will affect: (tick ☐ all the categories that apply)

	Protocol
○	Study objectives, design (including investigator's brochure, clinical activities, study length)
○	Study instruments, questionnaires, interview schedules
✓	Sample size
○	Recruitment methods
○	Eligibility criteria (inclusion and exclusion criteria)
○	Drug/device (composition, amount, schedule, route of administration, combination with other drugs/devices, safety information)
○	Data collection/ analysis
○	Principal Investigator. (Please attach revised conflict of interest and PI declaration statements. Refer: sections 7 and 8.4 in the New Protocol Application Form FHS013)

○	Consent form and information sheet
○	Recruitment materials (e.g. advertisements)
○	Administrative (e.g. change in sponsor's name, change in contact information)
✓	Other. Please specify: **This increased sample size will allow a more comprehensive review of the causes of premature ovarian insufficiency within our clinic population.**

4.1 In your opinion, will there be any **increase** in risk, discomfort or inconvenience to participants?	○ Yes	○ ✓ No
If yes, please provide a detailed justification/explanation:		

4.2 What follow-up action do you propose for participants who are already enrolled in the study?	
○	Inform current participants as soon as possible
○	Re-consent current participants with revised consent/assent forms (append)
○	No action required
○	Other. Please describe:

5. Detailed description of the change(s)

Please attach, for each amendment, a summary of all changes which clearly indicates:

i. Old wording (e.g. ~~strikethrough~~ text, CHANGED FROM and CHANGED TO)

ii. New wording (e.g. *italicized*, **bold**, tracked)

iii. Detailed rationale/ justification/ explanation for each change

6. Signature

My signature certifies that I will maintain the anonymity and/ or confidentiality of information collected in this research. If at any time I want to share or re-use the information for purposes other than those disclosed in the original approval, I will seek further approval from the HREC.			
Signature of PI	Johan de Spuy	Date	12.01.2017

Appendix 5. GSH Committee Consent to Access Folders

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
E-mail : Bernadette.Eick@westerncape.gov.za

Professor Z. van der Spuy
Obstetrics & Gynaecology
H-Floor - OMB

E-mail: zephne.vanderspuy@uct.ac.za

Dear Professor van der Spuy

RESEARCH PROJECT: Retrospective Review of Patient Folders

Your recent letter to the hospital refers.

You are hereby granted pro tem permission.

Please send along the following documentation:

- Ethics approval
- Annexure 2 (attached)
- Letter from HOD granting permission to perform research

Please send urgently to my office for further approval.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPEARTIONAL OFFICER
Date: 4 April 2016
BE/vms

C.C Mr L. Naidoo
Dr H. Aziz
Professor L. Denny

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