LISTER FERTILITY CLINIC
part of HCA Healthcare UK

Natural Killer (NK) cell tests – Patient Information

Introduction

Recurrent miscarriages and failure of implantation after transfer of good quality embryos in IVF treatment are the two major challenges in reproductive medicine. In the past decade considerable research efforts have been made to identify possible immunological causes for these challenges. However, currently the research is not able to provide a clear answer about the value of testing natural killer cells, particularly in the peripheral blood, nor whether different immunological treatment options available definitely improve the outcome of treatment cycles or pregnancies. This area of Reproductive Medicine remains experimental and controversial.

What is the evidence?

1. **RCOG** After a scientific review of the research available to date the Royal College of Obstetricians (RCOG) published a Scientific Impact Paper in December 2016 which states:

Uterine natural killer (uNK) cells form the major leucocyte population in the endometrium at the time of implantation and have received considerable attention in relation to their role in normal implantation and early placental development. Particular interest has been paid to their potential role in pregnancy pathology; specifically the role of uNK cells in recurrent miscarriage (RM) and recurrent implantation failure (RIF). Although several clinical studies have suggested that peripheral blood (PB) natural killer (NK) cells and/or uNK cells are increased in women with RM and RIF, data to date is inconclusive because of significant heterogeneity across studies arising from the use of different methods to quantify NK cells. An understanding of the role of these cells in reproductive failure and their value in clinical practice will not be established until a consensus is reached on how they should be measured.

Please see https://www.rcog.org.uk/globalassets/documents/guidelines/scientific-impact-papers/sip_53.pdf for a detailed assessment of the research to date and the RCOG conclusions about NK cell testing.

2. **HFEA** The HFEA's guidance regarding immunological testing is that there is no convincing evidence to support testing or treatment (see https://www.hfea.gov.uk/treatments/explore-all-treatments/treatment-add-ons/)

They state that there is no convincing evidence that a woman's immune system will fail to accept an embryo due to differences in their genetic codes. In fact, scientists now know that during pregnancy the mother's immune system works with the embryo to support its development.

Not only will reproductive immunology treatments not improve your chances of getting pregnant, there are risks attached to all these treatments, some of which are very serious. There are various different treatments associated with reproductive immunology, which are used to suppress the body's natural immunity, and all of which have risks:

- Steroids (e.g. prednisolone): Risks include high blood pressure, diabetes and premature birth.

- Intravenous immunoglobulin (IVIg): Side effects can include headache, muscle pain, fever, chills, low back pain, and rarely thrombosis (blood clots), kidney failure and anaphylaxis (a bad allergic reaction to the drug).

- TNF-a blocking agents (eg adalimumab, infliximab): Remicade is not recommended for use during pregnancy. Side effects can include infections including septicaemia, chronic infections such as tuberculosis, and severe allergic reactions to the drug.

- Intralipid infusions: Side effects include headache, dizziness, flushing, nausea and the possibility of clotting or infection.

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Background information

At the time of implantation, specialized embryonic cells (trophoblasts), which later form the placenta, begin to interact with the uterine lining (endometrium). When they meet with immune cells in the lining, they all become involved in a "cross talk" through mutual exchange of substances called cytokines. Because of this complex immunologic interplay, the uterus is able to help the embryo's successful implantation and, at the same time, protect the uterus from infection. It is therefore not surprising that failure of proper function of this immunologic interaction during implantation has been a focus for investigation as a cause of infertility, IVF implantation failure and recurrent miscarriage.

The most extensively studied group of immune cells are a type of lymphocyte (white blood cell) called natural killer cells (NK cells), they act as frontline in the protection mechanism of the body to attack infection and potential cancerous cells. After ovulation and during early pregnancy, Natural Killer (NK) cells comprise of more than 80% of the white blood cell population seen in the uterine lining. NK cells produce, as well as respond to, a variety of cytokines. It has been speculated that an imbalance of cytokines could have a negative impact on implantation or continuation of a healthy pregnancy.

The Lister Fertility Clinic has been involved in research in the area of natural killer cells and immunological aspects of recurrent failed implantation since 2003. We have concluded that a rise in the total value of NK cells has no effect on the outcome of pregnancy following IVF treatment. However, we have observed that an elevation in a specific sub-group of NK cells (CD-69) may be associated with a reduction in IVF pregnancy rates and possibly a higher miscarriage rate. We have published our experience in the medical journal *Human Reproduction*[1].
Other scientist's research work has also suggested that abnormally high activity of natural killer cells may have a negative impact on reproduction.

Failure of implantation, as well as recurrent miscarriages, is not due to a single problem and therefore it is unlikely to have a single solution. Although there is unlikely to be any one break-through that will completely change practice in the way IVF is done, a better understanding of the causes and treatment of miscarriages will gradually change the way we diagnose and treat recurrent miscarriages. Miscarriage has a tremendous emotional burden on the couples involved, but it is currently unclear whether immunological testing and treatment will help reduce the risk of miscarriage.

What are Natural killer cells (NK cells)?

NK cells are one of several types of lymphocytes in the immune system protecting us from infections by bacteria and viruses and possibly cancer cells; they are an important part of the defence system. There are several types of NK cells; some of them have negative impact on reproduction where as the others do not. NK cells are the most abundant immunological cells in the endometrial lining of the uterus, and can therefore come into contact with an implanting embryo. All the NK cells in the body are originated in the bone marrow from stem cells and

require the bone marrow microenvironment for complete maturation. After maturation the NK cell will either remain in the circulation to play a role of first line defence or they can migrate to various organs including liver, gastrointestinal tract, uterus where they undergo final maturation process and function as an immunological defence barrier in those tissues.

NK cell testing

NK cell can be tested in the blood or directly through a biopsy sample from the endometrium. The latter entails a minor operative procedure and analysis in a specialised laboratory. There seems to be some confusion regarding the type of test to be carried out. It is true that peripheral blood NK count may not be an accurate reflection of the environment inside the womb. There are some assumptions that an endometrial sample may be more accurate but there are also concerns about these tests. Every time a woman menstruates, that particular endometrium with all its NK cells are shed and therefore, in the next cycle, the number of NK cells may vary. Also, it is impossible to achieve a pure endometrial sample; if the sample is blood stained, the sample will contain endometrial lining as well as blood NK cells which will may reduce the accuracy of the results. There needs to be standardisation in the way that different laboratories measure uNK cells to improve the interpretation of data from studies.

Hence the uterine NK testing is not done at the Lister. Currently we have blood tests available to examine the activity of NK cells. It is important to note that although this test has evolved over the last few years, there is ongoing research in this area, interpretations of results can change in future and this area of testing and treatment needs to be regarded as experimental. In majority of cases of RM and RIF, it is the abnormal genetics of the embryos that cause failure to implant. It is important to be aware, that immune treatment will not help implantation of **genetically abnormal** embryos.

The HFEA, the Royal College of Obstetricians and Gynaecologists (RCOG), Science Advisory Committee and the American Society of Reproductive Medicine (ASRM) all agree that there is not enough evidence available at the moment to justify the blood tests, examinations (such as endometrial biopsies) and drugs that may be involved in testing and treatment.

"There is no conclusive evidence to show that these treatments are either beneficial or ineffective." HFEA

The HFEA "traffic light rating" for the use of reproductive immunology testing and treatments is "red", suggesting no good evidence to show that it is effective and/or safe.

You can read more about the HFEA traffic light system on fertility "add ons" in the information provided in your cycle packs or on the following link *https://www.hfea.gov.uk/treatments/explore-all-treatments/treatment-add-ons/*.

A fertility "add-on" is an "optional extras that you may be offered on top of your normal fertility treatment, often at an additional cost. They're typically emerging techniques that may have shown some promising results in initial studies but haven't necessarily been proven to improve pregnancy or birth rates."

INFORMATION FOR PATIENTS CONSIDERING TESTING FOR NATURAL KILLER CELLS IN PERIPHERAL BLOOD

***Please note**: The blood sample can be taken at the **Lister Fertility Clinic Monday – Thursday 09.30am – 13.00pm.** We are unable to take blood later than this as it has to be sent to a specialised laboratory.*

There are two parts of the NK cell blood test:

1. NK cells activation marker CD-69
2. NK cells cytotoxicity assay.

1. NK cell activation marker (CD-69)

This is a blood test to check the level of activation of NK cells in the circulation. Women may have large number of NK cells but if they are not activated, it is thought they will be unlikely to have a negative impact on reproduction. However, some women can have normal numbers of NK cells but the majority of them could be activated. Previous studies and our own research project suggested that elevation of the CD-69 activation marker of NK cells was associated with implantation failure and miscarriage. If the NK CD-69 is raised suppression with steroids may be tried therapeutically, although this has not yet been studied in a randomised controlled trial to demonstrate benefit of treatment.

2. NK cell cytotoxicity assay

This test is to check the sensitivity of the NK cells to foreign tissue and also the killing power of the NK cells. It involves culture of the NK cells with target cells (which are similar to pregnancy tissue) for a time period, and analysing the percentage of the target cells killed by the NK cells. If the NK cells are very sensitive to foreign tissue, and have high killing power, it will show a high percentage of the target cells have been killed. The second step in the cytotoxicity assay involves adding the available potential treatment options (IVIg, steroids or intralipid) to see if the killing power of the NK cells can be reduced by the medications. This part of the test helps to determine what treatment to offer the patient.

Which patients should consider NK blood tests?

Natural killer (NK) cell testing is NOT offered routinely to all patients starting IVF treatment at the Lister Fertility Clinic as we do not believe there is evidence to support this. We do not recommend performing these tests for all the patients prior to IVF treatment as the majority of the failed IVF cycles are likely to be due to other factors (eg embryonic, genetic, sperm, anatomical or endometrial factors) rather than immunological in nature. NK testing, may be discussed at consultation with a smaller population of patients who have a history of repeated failed IVF attempts with good quality embryos and/or repeated early unexplained pregnancy loss (miscarriage). It may also be discussed with patients with a history of endometriosis with previous failed IVF cycle/s, history of known autoimmune disorders (such as Lupus, Rheumatoid arthritis, Crohn's disease, ulcerative colitis, Ankylosing Spondylitis, Thyroiditis, Chronic fatigue syndrome).

Treatment options

There are 3 experimental treatment options for elevated NK cells:

1. **Steroid oral medications**
2. **IVIg (intravenous immunoglobulin-g) infusion**
3. **Intralipid infusion**

Literature suggests that the elevation of the NK cell activation marker and/or high cytotoxicity levels are associated with lower implantation or higher miscarriage rates follow IVF treatment. It has been speculated that this may be improved by immunotherapy treatment such as steroids or intravenous immunoglobulin (IVIg). Although an association with a successful outcome after IVF with IVIg or steroids[3-7] has been suggested, as yet there are no large randomised controlled trials to show a clear benefit from treatment as compared treatment without immunosuppressant. One study which combined the data from several smaller studies suggests there may be significant improvement in live birth rates following IVF when IVIg is used[6]. It is important to note that the immunological treatment is still experimental and not yet recommended by the Royal College of Obstetricians and Gynaecologists (RCOG) or HFEA.

1. Steroid (Prednisolone)

Prednisolone is an oral medication which can temporarily suppress the immune system and is used widely to treat a number of inflammatory conditions, even in pregnancy. Prednisolone is mostly inactivated as it crosses the placenta so transfer to the fetus is minimal.

Side effects

Side effects that can happen in the first few weeks of taking prednisolone include:

- water retention
- high blood pressure
- hyperglycaemia (high blood sugar) and glycosuria (sugar in the urine)
- increased susceptibility to infections
- heartburn
- behaviour disturbance e.g. nervousness / change in mood
- insomnia

Additional side effects associated with longer term use (ie several months)

- skin stretch marks (striae)
- osteoporosis
- myopathy (muscle weakness)
- Cushingoid feature consisting of moon face, buffalo hump, central obesity, increased tendency to bruising, acne and hirsuitism

Caution

Because prednisolone can cause high blood pressure, salt and fluid retention and high blood sugar, patients taking steroids for longer than 3 weeks will need to have their blood pressure, blood sugar level and full blood count checked every two weeks while taking steroids. These can be performed at the Lister Fertility Clinic or your GP.

Prednisolone should be used with caution if there is a history of ulcerative colitis, abscess or other bacterial infections, diverticulitis, peptic ulcers, hypertension (high blood pressure), congestive heart failure, history of blood clots, osteoporosis and Cushing's syndrome.

Prednisolone regime

Start taking Prednisolone 25mg by mouth daily with breakfast in the morning. The treatment is to start around day 7-8 of FSH injections in a stimulated cycle or day 10-11 in a frozen embryo transfer cycle and continued until the outcome of the pregnancy test is known:

If pregnancy test is **negative**, the Prednisolone can be stopped immediately as long as you have taken the prednisolone for 3 weeks or less.

If the pregnancy test is **positive,** you should continue until the end of 12 weeks of pregnancy (end of first trimester) after which the dose will be reduced gradually as detailed below. If you miscarry and have taken presdnisolone for more than 3 weeks you will need to follow the weaning off regime and we advise you to contact the Lister Fertility Clinic to speak to one of the nurses.

IMPORTANT: DO NOT SUDDENLY STOP PREDNISOLONE IF YOU HAVE BEEN TAKING IT DURING THE FIRST 12 WEEKS OF PRGNANCY. Please inform the doctor you are on prednisolone if you are admitted to hospital or need any surgical treatment while taking steroids.

Weaning off programme for prednisolone

If prednisolone is withdrawn too rapidly patients may experience nausea, fatigue, anorexia, dyspnea (difficulty breathing), hypotension (low blood pressure), hypoglycemia (low blood sugar), myalgia (muscle aches), fever, malaise, arthralgia, dizziness, sloughing off of skin and fainting. If you experience any of these problems, contact the clinic immediately.

The dose of the steroid is gradually reduced as follows:

- Prednisolone 20mg for 4 days then
- Prednisolone 15mg for 4 days then
- Prednisolone 10mg for 4 days then
- Prednisolone 5mg for 4 days then stop

2. Immunoglobulin-G infusion treatment (IVIg)

IVIg consists of concentrated and highly purified human immunoglobulins (antibodies), primarily IgG (immunoglobulin-G), prepared from pooled human blood donors. The dosage of IVIg and protocol will be determined based on the laboratory testing, patients weight and clinical response.

Side effects

Side effects to IVIg infusion tend to be related to the rate of infusion. Possible side effects include:

- Malaise (feeling unwell)
- a feeling of faintness
- fever or chills
- headaches
- nausea and vomiting

- shortness of breath, chest tightness, thrombosis and joint pains have rarely been reported.

Viral Safety

No cases of human immunodeficiency virus (HIV) transmission have been related to the administration of IVIg. However, there are still concerns about possible transmission of infectious diseases. It is recommended to use only IVIg products that have been prepared with an additional viral inactivation procedure. It is not yet possible to screen blood products for prions that cause Creuzfeld-Jacob disease (CJD or 'mad cow' disease), although the blood used to prepare IVIg is donated in USA where there is no known risk of CJD currently.

Hypersensitivity

Anaphylactic (serious allergic) reactions may occur during IVIg treatment in patients and therefore the patients are given antihistaminics during as well as after the transfusion.

Procedure

IVIg can be performed at Lister Hospital as a day case procedure or at home via Healthcare at Home. Please use the following procedure for booking the infusion:

At the Lister Hospital:

- call the IVF nurses, giving at least 1 working days notice to enable the IVIg infusion to be ordered via pharmacy.

NB. The infusion can be given on a weekend if necessary to your treatment cycle.

At home via Healthcare at Home:

- Call the medical secretaries 5 working days in advance so a referral can be made by your consultant.
- Healthcare at home will contact you to arrange delivery, take payment and arrange a date when the infusion can be given.
- Medication is delivered to your home address via a courier the day before treatment and an adult will need to be available to sign for this delivery.

NB. Healthcare at home do not perform infusions on the weekends.

Cost

The cost of IVIg treatment performed here at the Lister Hospital is approximately £1480 for each treatment. Up to 3 IVIg treatments maybe required.

1st IVIg treatment – *before or on day of vaginal egg collection (VEC)* = **£1480 (£980*** for Kiovig 10g x 2 & £500 Hospital bed fee (this fee is not charged if administered on the day of VEC))

2nd IVIg treatment – 4-5 weeks after first IVIg treatment (if positive pregnancy test) = £1480 **(£980*** for Kiovig 10g x 2 & £500 Hospital bed fee)

3rd IVIg treatment – 4-5 weeks after 2nd IVIg for ongoing pregnancies = £1480 **(£980*** for Kiovig 10g x 2 & £500 Hospital bed fee)

*Please note in the event Kiovig is out of stock, an alternative will be used as recommended by your consultant. You will need to enquire with the Lister Pharmacy for the specific cost.

The cost of IVIg treatment performed via Healthcare at Home is approximately £1,200-£1,400, depending on the number of doses needed.

3. **Intralipids**

Intralipid is an emulsion of soy bean oil, egg phospholipids and glycerin**;** it is licensed to be given to patients who are not able to take calories by mouth, to provide them with calories through an intra-venous infusion. This product is also used to provide certain nutrients (essential fatty acids) to people who have a fatty acid deficiency and the infusion may help to prevent, or reverse the signs of essential fatty acid deficiency such as scaly skin, poor growth and poor wound healing.

Some animal and human evidence has suggested that Intralipids (by providing the body with essential fatty acids that may help to lower the activity, or may normalise the cyto-lytic effect/killing power of Natural Killer cells in the blood) have a suppressive action on certain components of the woman's immune system, essentially safeguarding the embryo from the immune reactions which might otherwise result in implantation failure. Where NK testing has suggested that only Intralipid lowers NK cytotoxicity, it is a treatment option.

Worldwide there has been less clinical experience with Intralipid than with other immune altering agents such as steroids and IVIG and although patients have been successful following administration of Intralipid infusions, there remains no evidence of success from high quality trials and any benefit may be a possible placebo effect.

Also, the HFEA have confirmed that the President of the RCOG has expressed concerns about the administration of Intralipid infusion to women undergoing IVF and to those with a history of recurrent spontaneous miscarriage (RSM). The use of Intralipid infusion for these indications represents off-label use of the medicine and any doctors who prescribe this medication to infertility and RSM patients should take particular care to explain the possible risks of giving this infusion to a patient.

It is an infusion which is administered through an IV drip in the arm. It is administered by one of the nurses at the Lister or at home just like IVIg and the infusion will usually take about 4-5 hours.

Side effects:

Most patients experience no side effects from Intralipid infusions but due to the risk of allergic reaction to the ingredients it may not be suitable for patients who are allergic to soya bean oil or eggs.

Other side effects which have been reported occasionally include headaches, dizziness, flushing, and drowsiness, nausea, vomiting and sweating. As a precaution against allergic reaction, patients are given anti-histaminic medication.
The RCOG was particularly concerned after it was reported that a patient had developed a severe infection after being given an Intralipid infusion. However this infusion was not given in a hospital and so appropriate aseptic precautions may not have been used in preparing the infusion

Cost

The cost of intralipid infusions performed here at the Lister Hospital is approximately £550.88 for each treatment. Up to 3 treatments maybe required.

1st intralipid infusion – *before or on day of vaginal egg collection (VEC)* = **£550.88 (£50*** for infusion & **£500** Hospital bed fee (this fee is not charged if administered on the day of VEC))

2nd intralipid infusion – 4-5 weeks after first treatment (if positive pregnancy test) = £**550.88 (£50*** for infusion & **£500** Hospital bed fee)

3rd intralipid infusion – 4-5 weeks after 2nd infusion for ongoing pregnancies = £**550.88 (£50*** for infusion & **£500** Hospital bed fee)

*Please note in the event the intralipid infusion is out of stock, an alternative will be used as recommended by your consultant. You will need to enquire with the Lister Pharmacy for the specific cost.

For the cost of intralipid infusion performed via Healthcare at Home please contact them directly.

Before you can be given prescribed any of these medications, infusion your clinician must

1. *Ensure that you have been given appropriate information to read and consider the implications of taking this treatment.*
2. *Document in your case records that they have given you this information fact sheet.*
3. *After discussion, record in your case records the reasons for prescribing this off line medicine.*

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