

Natural Killer (NK) cell tests – Patient Information

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 efforts have been made to identify possible immunological causes for the mentioned challenges.

At the time of implantation, specialised embryonic cells (trophoblasts), which later form the placenta, begin growing into 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 growth 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 considered 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) is associated with a reduction in IVF pregnancy rates and it is a possible cause for a higher miscarriage rate. Other scientist's research work has also suggested that abnormally high activity of natural killer cells can 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 breakthrough 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 immune-based recurrent miscarriages. Miscarriage has a tremendous emotional burden on the couples involved, but with the further development of specific therapies, immunological miscarriages may become preventable.

We have published our experience in the medical journal *Human Reproduction*¹.

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. Previous studies suggest that overactive NK cells may cause damage to these embryos that are attempting to implant.

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 cells 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, however there is some good evidence on the link between peripheral blood NK activity and miscarriages and fertility issues. There are some assumptions that an endometrial sample may be more accurate but there are even more 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 not give accurate results.

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 and interpretations of results can change in future. Although some women have this as the main issue, in majority of cases, it is the abnormal genetics of the embryos that cause failure to implant. One needs 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

Please note: The blood sample can be taken at the **Lister Fertility Clinic Monday – Thursday 09.00am – 13.00**. 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 confirmed 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 trial.

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 have NK blood tests?

Natural killer (NK) cell testing is not offered to all patients starting IVF treatment. 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 embryonic rather than immunological in nature. NK testing, however may be discussed at consultation with patients who have a history of repeated failed IVF attempts and/or repeated early 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 and so on).

Treatment options

There are three 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 steroids³⁻⁷ 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 used⁶. It is important to note that the immunological treatment is still experimental and not yet recommended by the Royal College of Obstetricians and Gynaecologists.

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 hirsutism

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 prednisolone 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 PREGNANCY. 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 Creutzfeld-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 made from soya bean oil, egg yolk, glycerin and water. Intralipids 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.

Worldwide there is less clinical experience with Intralipids than with IVIg, but there is emerging data of its benefit and many patients have been successful following the treatment. Intralipid infusion therapy provides the body with essential fatty acids that help to lower the activity or normalise the killing power of Natural Killer cells in the blood thereby allowing the embryo to implant on the uterine wall. New research has suggested that women who have experienced recurrent miscarriages or multiple failed IUI or IVF cycles as a result of NK cell activation may benefit from such a treatment. There is evidence from both animal and human studies suggesting that intralipids administered intravenously may enhance implantation.

Many women who have suffered multiple IVF treatment/implantation failures and who are unable to afford more expensive treatment options turn to intralipid therapy. Other women who are concerned about taking IVIg (plasma transfusion) have also used intralipids as a treatment option.

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 Fertility Clinic or at home just like IVIg and the infusion will usually take about 4-5 hours.

Side effects:

Most of our 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.

Side effects such as headache, dizziness, flushing, drowsiness, nausea, vomiting or sweating may occur occasionally.

As a precaution against allergic reaction, patients are given anti-histaminic medication.

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.

Remember that your doctor has prescribed IVIg or Intralipids because they have judged that the benefit to you is greater than the risk of side effects. Most patients using this medication do not have any serious side effects

References:

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3. Scher J, Salazar C. Clinical experience with IVIg Rx in patients with prior failed IVF pregnancies: report of 30 consecutive patients.
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4. Coulam CB, Krysa LW, Bustillo M. Intravenous immunoglobulin for in-vitro fertilization failure.
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5. Ruiz JE, Kwak JY, Baum L, Gilman-Sachs A, Beaman KD, Kim YB, Beer AE. Effect of intravenous immunoglobulin G on natural killer cell cytotoxicity in vitro in women with recurrent spontaneous abortion.
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7. Fukui A, Fujii S, Yamaguchi E, Kimura H, Sato S, Saito Y. Natural killer cell subpopulations and cytotoxicity for infertile patients undergoing in vitro fertilization.
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CONSENT FORM

Patient's name:

Hospital No: X.....

Date of birth:

PART I: Consent to Blood test for Natural Killer cell assays

I have read the attached information leaflet about tests for Natural killer cells, treatment options and their side effects. I understand testing and treatment is currently experimental.

I agree to have blood taken for Natural Killer cell (NK cell) assays:

(i) CD69 activation marker YES / NO

(ii) cytotoxicity assay YES / NO

Signed

Date

PART II: Consent to treatment for abnormal NK blood test result

I have read the attached information leaflet about tests for Natural killer (NK) cells, treatment options and their side effects. I understand NK testing and treatment is currently experimental.

Having had the opportunity to discuss the results of my tests with a doctor I consent to the administration of

(1) Oral prednisolone YES / NO

(2) Intravenous infusion of immunoglobulin (IVIg) YES / NO

Signed

Date