Division of Medical Sciences and Graduate Entry Medicine, School of Medicine

Participant Information Sheet

Dear Potential Volunteer,

- You have been invited to take part in the research project: "*Novel Dynamic Proteomics Approaches to Investigate the Systems Level Pathology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)*".
- This document provides information about what is expected from you as a participant should you agree to take part.
- Please take time to read this document carefully.
- We encourage you to discuss it with friends and relatives before you make a final decision.
- Ask us if anything from this document is unclear, or you have any additional questions.
- If you decide to take part, firstly **THANK YOU**, and you will be given a copy of this document and your consent form to keep.
- Please also see the additional information about COVID-19-specific measures that will be in place to safeguard your participation.

We look forward to hearing from you.

Dr Daniel Wilkinson, Prof Philip Atherton, Prof Ken Smith, Dr Bethan Phillips, Dr John Williams, Dr Mathew Piasecki (Project Investigators).

Why is your participation in this research project important?

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is an extremely disabling condition, associated with many different symptoms, including post-exertional malaise. Any form of physical or mental exertion can lead to a worsening of symptoms.

ME/CFS can affect a person's ability to perform normal tasks, severely impacting on quality of life and independence. It is not well known or understood, even though it is currently estimated that ME/CFS is as common as more well-known conditions such as Rheumatoid Arthritis.

Even though there has been more research into ME/CFS in recent years, we still do not understand the causes. This makes accurate diagnosis very difficult, and the search for an effective treatment even more challenging.

It has been difficult to understand what causes ME/CFS, partly because it is a complex condition affecting a number of different systems, tissues and organs in the body. It often starts suddenly, with someone going from fit and healthy to bedbound in a matter of days. These factors can also make it difficult to identify what is causing the disease.

Proteomics

Because of the complexities surrounding ME/CFS, a new and novel approach is needed in researching the underlying causes. In recent years an analytical technique called proteomics has provided good promise in other conditions. It has helped to identify potentially unknown factors which may be contributing to the onset of disease.

Proteomics works by measuring the levels of several hundred to thousands of proteins present within a blood or tissue sample at the same time. Proteins are the compounds that make up our cells and help to control their function.

By looking at the differences between the levels of many proteins in someone with the disease compared to someone without the disease, we can potentially identify which proteins, and hence which pathways and/or tissues in the body, may be important in the development of the disease.

Standard proteomics techniques do not take into account the fact that the metabolic processes in our body are constantly changing, so that a single measure at one point in time may completely miss key changes important to the disease cause.

The aim of this project therefore, is to use a new type of proteomics technique called dynamic proteomics. Dynamic proteomics measures the levels of proteins in blood and tissue samples at several different time points, to capture important dynamic changes that are occurring in the metabolism of ME/CFS patients.

This may provide the detailed insight needed to understand which factors in the body control ME/CFS symptoms and disease progression. This would be a very important step in helping to improve quality of life for sufferers of this devastating condition.

Some immediate questions you may have.

Why have you been chosen?

- In order for this study to be successful we need to recruit 10 females with ME/ CFS who are aged 20 to 50 years, and 10 healthy females of a similar age.

Do you have to take part?

- No, participation is entirely voluntary. If you decide to take part, you are still free to withdraw at any time without giving a reason

What does my participation involve?

- You will be asked to attend the Medical School at the Royal Derby Hospital Centre for a number of study visits which are summarised below.

Study Visit	Details of Visit

<u>Screening Visit:</u> ***Aim of visit*:** To assess participant suitability for the study ***Approximate Duration of Visit:*** 1h	1. **A medical history will be performed.** 2. **Measures of Height and Weight will be recorded.** 3. **Assessments of heart function using ECG and Blood Pressure will be performed.** 4. **Eligibility for required exercise testing.** To ensure that the prescribed exercise testing does not induce too severe reaction that you are unable to complete the remainder of the testing, eligibility will be based on previously published criteria. You will be eligible to take part if you are completing clinician-prescribed regular low-intensity exercise (e.g., 10-min of gentle pace walking) without symptom exacerbation, and are physically capable of performing moderate-intensity aerobic exercise for at least 25 min. 5. **A small blood sample (~10mls) to measure health parameters, including liver and kidney function.**
Time to next visit: No more than 3 months after – 6 weeks average	
<u>Pre-study Assessment Visit</u> ***Aim of Visit*:** Collection of baseline data and samples and familiarisation with main study protocol ***Approximate Duration of Visit:*** 3-4h	1. **Baseline assessments of body composition using ultrasound.** An ultrasound machine uses soundwaves which humans cannot hear and provides information about structure underneath the skin and will be used to assess muscle size and structure. 2. **Measures of Symptom Severity.** In order to assess symptom severity we will ask you to complete a DePaul Symptom Questionnaire and clinical symptom data assessment. These assessments have been specifically developed to assess both CDC and CCC case definition criteria of ME/CFS. These assessments will be performed throughout the study period to monitor severity of symptoms as a result of the testing. 3. **Collection of baseline biological samples:** ∞ Single blood sample from an arm vein. ∞ A single muscle sample will be collected from the upper part (quads) of one leg. This will involve injecting some local anaesthetic to numb the area, and a making a small cut in the skin. Each muscle sample will be approximately the size of a pea. Biopsy sites will be closed by stitches, which are removed (and the wound site checked) after 5-7 days. This visit will be arranged with you at your convenience at the end of Pre-Study assessment visit.

Time to next visit: ~2 weeks	
<u>Initial Dynamic Proteomics Tracer Dosing Visit</u> ***Aim of Visit*:** To provide you with the stable isotope tracer deuterium oxide which will be used for the dynamic proteomics measures ***Approximate Duration of Visit:*** ~8 hours	1. **Provision of Deuterium Oxide Tracer:** Deuterium oxide is a metabolic tracer also called "heavy water". It is used so that we can measure the rate at which certain metabolic functions are occurring in your body, in this case the rate at which certain proteins are being made. It is perfectly safe to use and we have extensive experience of using this approach. You will be provided with 14mls/kg D2O to enrich the body water pool to ~1%. To mimimise the risk of dizziness from D_2O consumption (this can be a minor side effect of D2O consumption), this initial dose will be split into 6-8 smaller does provided throughout the day. Two hours after your final D_2O dose, you will be asked to provide a saliva sample (this can be done at home and stored in the fridge until returning to the lab). 2. Following consumption of D_2O tracer, you will be provided with tubes for collection of daily saliva samples (for monitoring of body water enrichment) and daily D_2O tracer tops (~10% of initial dose, required to maintain body water enrichment at 1%) to be consumed each day for the next 2 days. **3.** For the next 2 days additional blood samples will be collected via venepuncture at the participants home by a

Time to next visit: 2 days	
<u>Exercise Stress Test and Assessment Visit 2</u> ***Aim of Visit*:** Collection of additional study measures and biological samples, and performance of an exercise stress test to elicit fatigue ***Approximate Duration of Visit*:** ~3-4h	1. **Repeat of Baseline Assessment measures:** Ultrasound, cognitive and motor control tests. 2. **Performance of exercise stress test.** This exercise test is designed to influence a degree of post-exertional malaise. The protocol chosen is based on previously published work. You will be asked to complete 25mins of cycling at an intensity set at 70% of age-predicted maximal heart rate. Exercise will begin at a low intensity (20 watts) and gradually increased until target heart rate is achieved and maintained for 25mins, with a 3 min active recovery period. Your ratings of perceived exertion and leg muscle pain will be monitored every 5mins. **3. Collection of additional biological samples:** ∞ Single blood sample from an arm vein. **4.** A single muscle sample will be collected from the upper part (quads) of one leg using same procedure as before. **5.** Collection of a single CSF sample via lumbar puncture. Lumbar puncture will be performed by Dr Williams, a consultant anaesthetist and group clinical lead, who has over 20y experience of performing this procedure. A small needle will be inserted between two of the bones of the lower back, which will allow the collection of a small amount of CSF. More detail on this procedure has been provided in an appendices at end of this document, including procedure, risks and any side effects that may occur. This will also be provided to you on the study day for you to familiarise yourself with again before sampling. 6. For the next 5 days following this visit additional daily blood samples will be collected via venepuncture at the participants home by a visiting phlebotomist, who will also collect daily saliva samples. You will be asked to continue with consuming your daily D_2O top ups as well.

Time to next visit: 5 days	
<u>Post Study Assessment Visit</u> ***Aims of Visit*:** Collection of final study measures and biological samples. ***Approximate Duration of Visit*:** ~3-4h	1. **Repeat of Baseline Assessment measures:** Ultrasound, cognitive and motor control tests. 2. **Repeat of biological sampling:** ∞ Single blood. ∞ Single Muscle biopsy. ∞ Single CSF sample via lumbar puncture.
Time to next visit: 1 week	
<u>Post Study/Exit Visit</u> ***Aims of Visit*:** Participant evaluation and wound checks ***Approximate Duration of Visit*:** ~1h	1. **Biopsy and Wound Site Check and Post Study Monitoring**: Biopsy and wound sites from the post-study assessment will be checked and sutures removed at this visit. You will also be invited to return a post-study participant feedback form at this visit (anonymous) which will be given to you on your post-study assessment. The inconvenience allowance for this study will also be arranged at this visit.

To avoid and minimise potential non-exercise induced PEM, the following adaptations and additions to the above protocol can be included where requested or needed:

- All unnecessary travel will be avoided where possible, however where needed you will be provided with pre-booked taxi transport to bring you to the research facility.

- Initial screening (excluding ECG, BP and blood sampling) will take place at your home or remotely (phone or via video conferencing application) if requested.
- The tracer administration and stitch removal can be performed at your home home where possible to avoid any unnecessary exacerbation of PEM symptoms.
- To minimise PEM for any on-site visits, you will be provided with regular rest breaks in designated rooms which are adapted to the needs of the volunteers condition. These will be discussed with you at screening as we understand that the ME/CFS is a very individual condition, one thing that might be comfortable for one volunteer, may not be for another. Individual measures can be put in place to minimise discomfort as needed.

For ease of interpretation, a summary table of all procedures is provided below, any concerns over scheduling can be discussed with the researchers at screening.

Activity		**Up to 3 months break (average 6 weeks)**		**2 week break** (with one visit for wound check on day 5)										**1 week break**	
Medical history	✓														
Height & weight	✓														
ECG & Blood pressure	✓														
Body composition			✓					✓						✓	
Cognition & motor control			✓					✓						✓	
Lumbar puncture								✓						✓	
Wound check														✓	✓
Muscle biopsy			✓					✓						✓	
Drink D2O					✓	✓	✓		✓	✓	✓	✓	✓		
Saliva sample			✓		✓	✓	✓		✓	✓	✓	✓	✓		
Exercise test			✓					✓							
Blood sample	✓		✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	
Day:	**0**		**1**		**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**30**
Visit:	**Screening**		**Pre-study assesment**		**Initial tracer dosing**	**At home**		**Exercise test & assessment**	**At home**					**Post study assessment**	**Post study/exit**

What is the drug or procedure that is being tested?

No drug or procedure is being tested and all procedures used are regularly performed within the research group.

What are the side-effects or risk of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known. However, we have outlined the potential risks associated aspects of this study below.

Procedures

Venepuncture for blood sampling has a risk of bruising but is a procedure that will be carried out only by trained staff.

Muscle biopsies are performed under local anaesthetic by a doctor trained in this technique. In all cases there is a slight risk of infection at biopsy sites but this will be minimized by the use of sterile techniques and dressings. All wounds will be monitored for 7 days for any sign of infection. Some muscle tenderness, stiffness and bruising may be felt for 2-3 days afterwards but simple painkillers usually effectively abolish this. There is a slight risk of damaging cutaneous and other soft tissue nerves during biopsy techniques, but this is rare and normal nerve sensation normally returns within a few months. It is common for a biopsy to leave a scar, however in most individuals a biopsy scar is very small and fades with time to near invisibility. We have been performing muscle biopsies in Derby for over 15 years using this technique and have found they are very well tolerated.

Lumbar Puncture

Used to collect cerebrospinal fluid from your lower spine. Samples are collected under local anaesthetic by a doctor with over 20 years experience of performing this procedure. You will remain within the unit in recumbent position for 1h to monitor for any possible complications, although complications are rare, there are possibilities that you may suffer a headache which occurs in 1:100 people. See Lumbar Puncture supplementary information below.

Tracers

The heavy isotopes used within the **tracer compound D_2O** are naturally occurring and non-radioactive, and are completely safe for application in human research. As a research group we have over 30 years' experience in applying heavy isotope tracers to human research. There is a slight risk of dizziness associated with consuming D_2O, however this is reduced by splitting the total dose into several small dosings over a number of hours, as is being done here

The exercise being performed will likely lead to symptoms of post-exertional malaise, and there is the risk that it could lead to a worsening or relapsing of symptoms in some individuals. To avoid this we will continuously monitor symptom severity throughout, and have designed the exercise protocol based on previously published work in this field. To minimise worsening of symptoms following completion of this study we will implement eligibility criteria (described above) to ensure that all volunteers are aware and capable to performing the ascribed exercise protocol. If you think that this kind of exercise will likely be too severe for your condition, please inform the researcher.

What are the possible benefits of taking part?

Participation in the study will provide you with some information about your muscle structure and function from the ultrasound scans and functional assessments, as well as your fitness.

Overall it is hoped that the information we gain from this study will give us a better understanding of the underlying causes of ME/CFS, which could in the future assist with the development of improved diagnostics and treatment.

Each volunteer, following completion of their testing will be provided with a summary of their own personal data in the hope that will assist them personally in managing their disease

You will also receive £400 inconvenience allowance on completion of the study.

What are the possible disadvantages and risks of taking part?

It is possible that the routine tests could detect unknown health problems. Examples of these include diabetes and high blood pressure, as they are common and often undiagnosed. Should this be the case, you will be informed and advised to attend your GP practice for further management. Your GP will also be notified.

As one of the primary measures of the study realtes to PEM, there is the risk that participation may lead to an increase in your symptoms as a result. To avoid any unnecessary exacerbation outside of the prescribed testing protocols, additional measures specific to each individual will be introduced following discussion with you at screening as described above. Symptoms will be assessed regularly throughout the study to monitor severity. Furthermore, PEM triggers will be discussed in detail with you prior to participation to ensure that if PEM is triggered it is not too great in severity and that you have sufficient and adequate recovery time as to avoid permenant worsening of symptoms.

What are the exclusion criteria for this study?

For this study we are recruiting:

i. healthy female volunteers aged between 20 and 50 years, who are of normal weight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid an inconvenience allowance or having an invasive procedure (e.g. blood sample >50ml), you would not be eligible to take part.

ii. female volunteers suffering from ME/CFS of the same age as outlined by the 1994 CDC-Fukuda and 2003 Canadian case definitions for ME/CFS.

You would also be unsuitable if you have particular medical conditions (such as neurological conditions) or are taking certain medications. If you are interested in this study, please discuss these further with the research team.

What if something goes wrong?

- If you suffer any symptoms or side effects, you should report them immediately to the study research team (see end of sheet for contact details).
- If you have a concern about any aspect of this study, you should ask to speak to the study team who will do their best to answer your questions.
- The researchers contact details are given at the end of this information sheet. If

you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator Dr Daniel Wilkinson.

- If this achieves no satisfactory outcome, you should then contact the FMHS Research Ethics Committee Administrator, Faculty Hub, Medicine and Health Sciences, E41, E Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH or via E-mail: FMHS-ResearchEthics@nottingham.ac.uk. Please quote ref no: FMHS 205-0221.

Will my taking part in this study be kept confidential?

We respect your right to privacy, and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement, will carry details of your name and address, but no health-related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to University staff with direct responsibility for ensuring the study is conducted appropriately.

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the study site will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies (if you agree to this). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time precautions will be taken by all those involved to maintain your confidentiality and only direct members of the research team will have access to your personal data.

You can find out more about how we use your information and read our privacy notice at: https://www.nottingham.ac.uk/utilities/privacy.aspx

What will happen to any samples I give?

We will seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?

In the current study we will not be using your samples for genetic testing.

What will happen to the results of the research study?

Data collected during the study will be published in the scientific literature enabling other health professionals to use the information, it may also form part of an education thesis. You will not be identified in any publication. There is usually a delay of up to one year from the study completion before this occurs. Should you wish to be informed of publications resulting from this study, please inform the study team.

Who is funding the research?

This study is funded by the US Department of Defense Discovery Award.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee.

Contact for Further Information

Thank you for your interest in this study. For further information please contact: Daniel Wilkinson, d.wilkinson@nottingham.ac.uk, contactable on 01332 724850.

The research team is alternatively contactable at The University of Nottingham, Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, Royal Derby Hospital, Derby, DE22 3DT.

Supplementary Information

1. Covid-19 for Human Research Volunteers

Following the recent outbreak of Covid-19 infection in the UK, close contact between people outside of family groupings or 'support bubbles' is being discouraged to reduce the spread of the virus, and to protect vulnerable individuals from contracting Covid-19.

It is important for you to know that during this research study, it will not always be possible to maintain recommended social distancing between researchers and yourself. Research staff will be required to be close to you when performing certain tasks, including: measuring blood pressure and recording an electrocardiogram (ECG) during the screening visit, and taking blood samples, muscle biopsies or CSF samples during your study visits.

Government guidelines are constantly evolving in response to changes in the Covid-19 situation, and procedures we employ during this study will therefore be changing in line with these. However, currently we will be implementing the following measures to protect you and the researchers working on this project.

1. Please do not attend any scheduled study sessions if you, or anyone in your social unit, is displaying (or has displayed within the previous 14 days) signs of COVID-19 infection*, or if you are self-isolating following contact with someone who has displayed signs of infection. We will contact you the day before each time you are due to attend the University to check your symptom and isolation status.
2. Similarly, study researchers will not enter University buildings if they, or anyone in their social unit, is displaying (or has displayed within the previous 14 days) signs of infection or if they are self-isolating following contact with someone who has displayed signs of COVID-19 infection.
3. Social distancing will be observed at all times where possible.
4. To keep the number of people within University buildings to a minimum, please do not bring additional people (i.e., siblings or partners) to any study session.
5. The number of individuals (researchers and participants) in study areas will be kept to a minimum, with all individuals required to maintain good hand hygiene whilst in the units and to wear face coverings at all times. As such, on arrival, you will be i) asked to cleanse your hands thoroughly at a hand-wash station and/or using alcohol gel. In addition, ii) if you forget to bring your own face covering with you, you will be provided with a disposable one to wear during your visit.
6. Prior to your arrival, all areas that you may touch will be cleaned.
7. At the end of the study visit, you will be asked wash your hands thoroughly again before leaving.

*Please visit: https://www.nhs.uk/conditions/coronavirus-covid-19/symptoms/

2. Lumbar Puncture

Supplementary Information – Lumbar Puncture Procedure – taken from https://www.nhs.uk/conditions/lumbar-puncture/

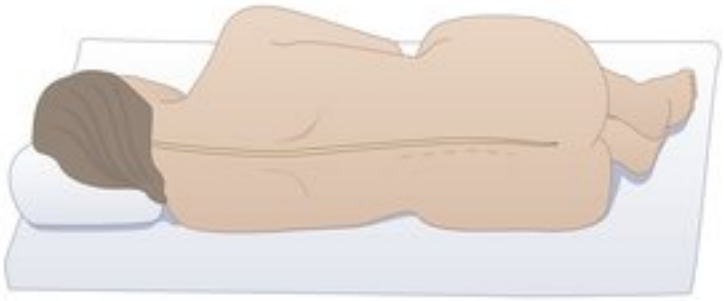
A lumbar puncture is where a thin needle is inserted between the bones in your lower spine. It should not be painful, but you may have a headache and some back pain for a few days. It's carried out by a doctor.

Before having a lumbar puncture

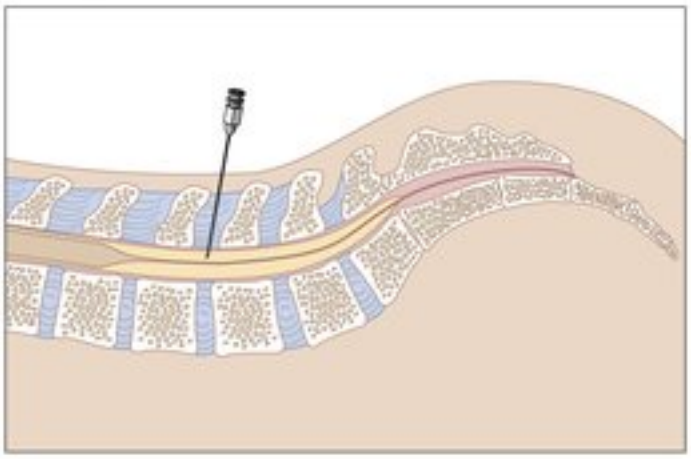
Your doctor should explain what's going to happen. On the day:

- you'll usually need to undress and change into a hospital gown before the procedure – you might also want to use the toilet

What happens during a lumbar puncture



You normally lie on your side, with your legs pulled up and your chin tucked in



This allows the needle to be inserted between the bones more easily

The doctor will:

1. Clean your skin and numb the area with local anaesthetic (you'll be awake during the procedure).
2. Insert a thin needle through the skin, between 2 bones in the lower part of your spine. This should not be painful, but you may feel some pressure.
3. Remove the needle once the procedure is finished and apply a small plaster or dressing.

How long does a lumbar puncture take?

A lumbar puncture takes around 30 to 45 minutes, but you'll need to stay lying down under supervision for at least another hour.

You'll be able to go home as soon as you feel ok to do so, but you would not be able to drive yourself home, a taxi will be provided for you

Side effects of a lumbar puncture

A lumbar puncture is generally a safe procedure and serious side effects are uncommon.

The most common side effects are:

- headaches, which can last for up to a week
- swelling and lower back pain where the needle was inserted – this should get better on its own after a few days and is normally nothing to worry about

Recovering from a lumbar puncture

While you're recovering from a lumbar puncture:

Do:

- ✓ drink plenty of fluids
- ✓ take painkillers, such as paracetamol
- ✓ lie down instead of sitting upright
- ✓ try drinks containing caffeine, such as coffee, tea or cola – some people find this helps to relieve the headaches
- ✓ remove the dressing or plaster yourself the next day

Don't:

- ➢ drive or operate machinery for at least 24 hours
- ➢ play sport or do any strenuous activities for at least a week

Non-urgent advice: Contact the hospital team or a GP if:

- your headaches are severe or do not go away
- you're feeling or being sick
- you have a very high temperature or feel hot and shivery
- it's painful to look at bright lights
- the swelling in your back lasts for more than a few days or keeps getting worse
- you see blood or clear fluid leaking from your back.