Standard Operating Procedure

Blood Sampling and Anthropometry
in
WP2 Task 2.2 Endocrine Dysfunctions, HARMONIC

Version: 1.0

Date of validity: (First patient inclusion)
Valid until: (Last patient inclusion)

Kommenterede [MW1]: Must be added

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Date:	Date:	Date:
Signature:	Signature:	Signature:

Table

1. Objectives

The purpose of this Standard Operating Procedure (SOP) is to ensure the procedure of blood sampling and anthropometry to obtain endocrine parameters under standardized conditions within the HARMONIC study. It is valid for the blood collection and measurement of the first included patient before, during and after external beam radiotherapy (EBRT) and loses its validity after the last included patient who's EBRT has been completed (see WP2 protocol for inclusion and exclusion criteria).

2. Applicable documents

- WP2 Study Protocol, Version xxx
- SOP data entry in clinical database
- Laboratory manual?!

Kommenterede [MW2]: Is there actually already an SOP?

Kommenterede [MW3]: Is the SOP of biology already finished?

3. Responsibilities/Partners

- Prof. Beate Timmermann, Clinic for Particle Therapy – University Hospital Essen (Germany)
- Dr. Stephanie Bolle, Dr. Brice Fresneau – Gustave Roussy (France)
- Dr. Yasmin Lassen, Dr. Sonja Karabegovic – DCPT, Aarhus University Hospital (Denmark)
- Dr. Gilles Defraene, Dr. Karin Haustermans – KU Leuven (Belgium)

4. Performance – Blood Sampling

4.1. Time Frame of Collection

See Annex I c.

4.2 Requirements

4.2.1 General and Special

a) General / Inventory / Devices

Equipment of the blood sampling for **each patient**

Table 1 Required Supplies: Blood sampling (General)

Amount	Equipment
1	Roller stool (for the examiner)
1	Hospital bed or blood collection chair with armrest (for the subject)
1	Armrest (washable)
1	Special waste container / Sharps disposal box (1 in use, 1 replacement)
1	Infectious waste biohazard bins
1	Tourniquet
2	Test tube rack (for the filled blood collection tubes)
1	Vomit bowl (for single use)
1	Radio controlled clock

b) Special supplies

Table 2 Required Supplies: Blood sampling (Special)

Amount	Supply
2	Winged blood collection set (butterfly devices) (different sizes)
1	Antiseptic skin disinfection spray before blood sampling
1	Surface disinfection (devices, arm rests, work surfaces)
1	Cellulose swabs with dispenser
2	Plaster
1	Pack of non-sterile gloves (different sizes)
1	Package garbage bags
1	Pack of tissues
	Subject specific label set from the respective hospital/laboratory XY (adapt to local conditions!)

4.2.2 Inventory

a) General / Inventory / Devices

- Refrigerator (4°C) with deep-freeze section (for freezer packs) for sample storage until analysis in the laboratory. Alternatively, suitable transport facilities can be provided. It must always be ensured that the cooling chain is not interrupted.

b) Special supplies

- Tubes (according to the specifications of the respective laboratory) with cap

4.2.3 Documents

- Latest version of *Blood Sampling and Anthropometry in WP2 Task 2.2 Endocrine Dysfunctions, HARMONIC*
- Laminated paper labels for blood tubes with sample ID
  -> CAVE: Partner participating in WP5

Kommenterede [MW4]: Check with WP5 and adjust SOPs if necessary.

4.2.4 Information

- **Patient information**
  - Local patient ID label is to be attached to the patient's medical record and matched with the name of the actual patient, before processing, in the direct interview of the patient.
- **Sample information**
  - Available samples ID number.

4.3 Purpose, Sample Preparation, Sample Collection, Sample Processing

4.3.1 Purpose

Blood sampling within the scope of Task 2.2 Endocrine Dysfunction, summarized in Table 3, serves as laboratory chemical determination of endocrine parameters, the results of which will be incorporated into HARMONIC's clinical database and contribute to answering scientific questions.

4.3.2 Sample Preparation

Table 4 summarizes the essential parameters in subject preparation and sampling and indicates the internationally recommended laboratory medical guidelines and their principles.

Sarstedt serum-gel Monovettes are used to determine hormone parameters for the patients. These tubes contain kaolin-coated polystyrene beads for coagulation activation. The separating gel (polyacrylate) in the serum-gel Monovettes is now standard in most laboratories due to its direct use in analyzers.

The blood sampling in a sitting or lying position has been retained in the current procedure (sitting posture). According to the Joint EFLM-COLABIOCLI Recommendation for venous blood sampling (2018), pre-sampling can also be performed in lying position. It is recommended that the patient should not change his/her body position 15 minutes before the blood sample is taken. *If a change in posture is unavoidable within this time period, it should be documented to allow correct interpretation of test results. If a patient has properly rested for 15 min in the waiting area, a short walk from the waiting area to the collection area is considered to be acceptable and does not need to be documented [1].*

There is no technical difference between taking blood samples from infants and older children, adolescents or adults. The blood sampling equipment only has to be adapted to the small dimensions of the vessels. In principle, a calm and friendly atmosphere is very important for the young patients. Children cooperate much better if the procedure is explained (depending on age). The application of a local anesthetic patch to the anticipated area about one hour before puncture can ensure a harmonized procedure. Placing the patient on the lab of mother, father or assistant can also help to simplify the situation. It is very important that the arm is well fixed in place, as reflective escape movements must be expected in all cases.

Avoid taking blood from an existing central venous catheter (cvc) as this may lead to contamination of the blood sample or false results. It is acceptable, but not ideal, to take blood samples if an intravenous device is inserted first before the cannula is connected to the fluids needed for treatment. In some cases (especially in very young children), it may be appropriate to draw blood through the cvc. According to the literature, there are three methods available: Discard, push-pull (mixing) and reinfusion [2]. For reasons of uniformity, blood should be drawn using the discard method. It is presumed that the discard procedure is well known. Further information can be found in Annex I e under "Central Venous Catheter Blood Draws - Discard".

Table 3 Type and purpose of use

Institute	Material type	Laboratory	Sample (mandatory)	Sample (optional)
University Hospital Essen	2 Serum, 1,2 ml Monovette and 1 Blood Count 1,6 ml Monovette	Central Laboratory, University Hospital Essen	IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron	ACTH
Gustave Roussy	2 Serum, 1,2 ml Monovette and 1 Blood Count 1,6 ml Monovette		IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron	ACTH
DCPT, Aarhus University Hospital	2 Serum, 1,2 ml Monovette and 1 Blood Count 1,6 ml Monovette		IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron	ACTH
KU Leuven	2 Serum, 1,2 ml Monovette and 1 Blood Count		IGF-1, GH, LH, FSH, TSH, fT3	ACTH

Kommenterede [MW5]: Please complete the table with the name of your laboratory

	1,6 ml Mono-vette		fT4, Estrogen, Testosterone, Progesteron	

Table 4 Recommendations on venous blood sampling – Pre-sampling and sampling

Parameter	**Recommendation**
Verify patient is fasting and properly prepared (sobriety)	For the lab-chemical analysis of hormones in blood, the sobriety of patients is not necessary.
Time of blood sampling	Unitary in the same time window, usually in the morning, 7-9 am (consider the circadian variation)
Rest period in unchanged body position before blood collection	About 15 min
Body position during blood collection	The sitting position is recommended for outpatient blood collection
Duration of blood stasis before blood collection	Max. 1 min - open as soon as needle in vein
Blood collection technique	- Large volume cannula - No bathing of the arm in hot water - No prolonged searching/ 'poking' with the needle on of a job - No forced/rapid aspiration - No aspiration of para-venous blood - No repeated fist closure/pumping
Order of draw	First serum, then EDTA if required

4.3.3 Sample Collection – Examination Site

The room temperature should be comfortable (about 20°C-24°C). The blood collection chair can be bent over to form a bed, so that the test person can lie down in case of indisposition.

The material stock at each blood collection site should be checked daily before starting the blood collection. The blood collection area must always be kept thoroughly clean. Used material must always be disposed immediately in the appropriate containers. Clean the table, the armrest and, if necessary, the floor after each blood collection with surface disinfection and disposable tissues/cellulose.

Thorough hand washing or hand disinfection must be performed before and after each blood sampling. The examination site must be staffed by a technically experienced examiner who is experienced in blood collection. The qualification must be documented in a training and confirmed by the study management.

4.3.4 Sample Collection – Preparation

- In general, a physician or qualified person must be able to personally reach the study center within a reasonable time (note circadian variation). The patient is accompanied to the blood collection site. After taking off the sweater/jacket, the test person should already be seated on the blood collection chair with armrest (or hospital bed) at this stage to avoid getting up again before the actual blood collection. It should be noted whether the blood sample was taken lying down or sitting. If another examiner collects the blood, the record of the patient is handed over to him/her.

- Conversation with the patient:
  - Explain your intention to the patient.

- Give the patient the opportunity to ask questions. Give him/her the impression that his/her questions will be taken seriously and answered competently.
- If the patient is a little anxious, take time, try to calm him/her down and convince him/her of the importance of the examination.
- Do not under any circumstances try to persuade the patient to take a blood sample.

- Carefully check that the collection number of the blood collection tube matches the collection number in the patient record.

- Prepare the other material required for blood collection as listed in Table 1.

4.3.5 Sample Processing

Profound knowledge regarding blood sampling is required. Detailed information about the process can be obtained in Annex I d.

4.4 Post Sampling

After making sure that the patient is well, clean the workplace and accompany him/her to the next examination. Used swabs, cannula and other items are disposed into the appropriate containers. In order to minimize the risk of injury, it is mandatory to avoid recapping of protective caps on cannulas [5]. Finally, clean the armrest, work surface, blood collection chair or, if necessary, hospital bed with a surface disinfectant and disposable cloths. Thereby please pay attention to the local regulations. Throw away the used disposable cloths in the container assigned for this purpose. Please label the Monovettes and store the samples in a refrigerator or a suitable cooling medium until collection or transport to the laboratory.

5. Anthropometry

5.1. Time Frame of Measurements

See Annex II a.

5.2 Requirements

5.2.1 Supplies

a) Devices/Inventory

Amount	Equipment
1	Roller stool (for the examiner)
1	Metric Stadiometer
1	Measuring tape (in cm); suitable for cleaning
1	Surface disinfection (devices, work surfaces)
1	Pack of non-sterile gloves (different sizes)
1	Package garbage bags
1	Pack of tissues
1	Hospital bed/Couch (for very small patients)

5.2.2 Documents

- Latest version *of Blood Sampling and Anthropometry in WP2 Task 2.2 Endocrine Dysfunctions, HARMONIC*

5.2.3 Information

- **Patient Information**
  - Local patient ID of medical record has to be matched with the name of the actual patient, before processing, in the direct interview of the patient.

5.3 Purpose, Preparation, Performance

5.3.1 Purpose

The evaluation of sitting height, abdominal girth and hip size in children and adolescence (<18 yr.) with head and neck tumors should provide additional information (besides body height and weight) on the functionality of the endocrine glades, with focus on the thyroid, hypothalamus and pituitary glade during and after radiotherapy.

5.3.2 Preparation

Measuring devices used on more than one patient pose a risk of infection and should be cleaned after use in accordance with local guidelines. Patients who have an infection but meet the inclusion criteria will also need to have their sitting height, abdominal girth and hip size measured; local infection and prevention measures should be used as precautions in these circumstances.

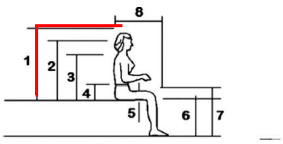
Non-sterile gloves are not routinely required for this procedure. The examiner must assess each patient for the risk of exposure to blood and body fluids [6] (Royal College of Nursing, 2018) and be familiar with local guidelines for glove use.

5.3.3 Performance

Please check the patient's identity on the basis of the patient file. Explain the procedure to the patient and allow time to ask questions. Then obtain verbal informed consent. Evaluate the patient's mobility and ability to stand unaided, then choose the appropriate method for measurement. Make sure that the equipment has been cleaned and decontaminate your hands according to local guidelines. An apron should be worn in case the patient needs physical help to get up from bed or chair.

a) Sitting height

The sitting height is measured from the crown of the head to the seat of the patient (see figure 1). The patient sits in an upright position on a flat stool with his/her spine facing the stadiometer. Curving of the back should be avoided as far as possible. Ask the patient to take a deep breath during the measurement and hold it for a short moment.



1	Sitting Height
2	Eye Height
3	Shoulder Height
4	Elbow Rest Height

Figure 1 Measurement of sitting height

For very young patients the crown-rump length is determined. Use a measuring tape for this. Place the infant on the side of a couch/hospital bed and start measuring from the crown to the tailbone. Please make sure that the small patient is as straight as possible. If necessary, you can ask a parent or assistant for help [7] [3]. Document patient's sitting height to the exact cm, in the patient file.

b) Abdominal girth

The abdominal girth is measured with a measuring tape at umblical level. Please ask the patient to uncover his or her abdomen and make sure that the patient is in a straight position (standing or sitting). Place the measuring tape at umbilical level and lead it horizontally around the belly (see figure 2, A). Also make sure that the measuring tape is straight and not too tight around the patient [4]. In patients <2 years of age it may be appropriate to include an assistant or parent. Document patient's abdominal girth to the exact cm, in the patient file.

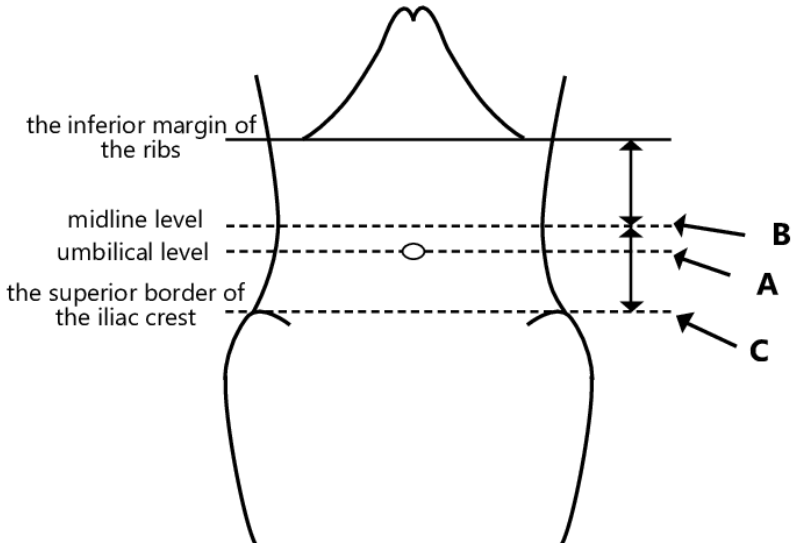


Figure 2 Measurement of abdominal girth and hip size

c) Hip size

For hip measurement, the measuring tape is placed at the level of the superior border of the iliac crest and is guided horizontally (not too tight) around the level (see figure 2, C). Please make sure that the measurement tape is guided as straight as possible to avoid incorrect measuring results. Document patient's hip size to the exact cm, in the patient file.

5.3.4 After Measurement

After taking the values, clean the workplace and accompany the patient to the next examination. Clean the measuring tape, work surface, metric stadiometer and if necessary, the hospital bed/couch with a surface disinfectant and disposable cloths. Thereby please pay attention to the local regulations. Throw away the used disposable cloths in the container assigned for this purpose.

6. Response to deviations

Identification of patient

Unconfirmed patient identification can lead to missing traceability of the sample and to sample mix-ups. If there is any uncertainty about the subject or the sample, the sample should be discarded.

Local conditions and equipment

For blood collection, it must be checked whether clean and hygienic conditions are present. If there are any deviations, the sampling process must be stopped and the responsible person contacted in order to take corrective action and release the process again.

Storage and transport conditions

Storage and transport conditions must be controlled to ensure clean and hygienic environmental conditions for products, test samples and labels. In case of deviations, sampling is interrupted and the responsible person must decide on corrective measures as well as a subsequent release of the process.

Annex

I. Blood Sampling

a. Recommendations on Venous Blood Sampling – The Order of Steps

	Step	Strength of evidence
1.	Identify a patient	1C
2.	Verify patient is fasting and properly prepared (not necessary for hormone analysis)	1B
3.	Obtain supplies required for blood collection	2C
4.	Label/identify tubes	1C
5.	Put on gloves	1C
6.	Apply tourniquet	1A
7.	Select venepuncture site	1B
8.	Clean sampling site	1B
9.	Puncture the vein	1A
10.	Draw first tube	1A
11.	Release the tourniquet	1A
12.	Gently invert the tube once (one full inversion)	1B
13.	Draw additional tubes following order of draw	1B
14.	Remove needle from the vein and activate safety feature	1A
15.	Dispose of the needle	1A
16.	Bandage the puncture site	1C
17.	Tell a patient to apply a gentle pressure for 5–10 min and not to bend the arm	1C
18.	Invert all tubes 4 times	1B
19.	Remove gloves	1A
20.	Advise patient to rest for 5 min and ensure bleeding has stopped before leaving the site of venous blood collection	1B

1A = Strong recommendation, high quality evidence; 1B = Strong recommendation, moderate quality evidence; 1C = Strong recommendation, low quality evidence; 2C = Weak recommendation, low quality evidence . [1]

b. Parameters and Units

Parameter	Unit
IGF1	ng/ml
GH	ng/ml
ACTH	pg/ml
LH	IU/l
FSH	IU/l
TSH	mU/l
fT3	pmol/l
fT4	pmol/l
Estrogen	pg/ml
Testosterone	nmol/l
Progesteron	ng/ml

c. Time Frame of Collection

The following table shows the period of the parameters to be collected. The table also shows for each parameter the level of importance.

	Time points			
Parameter	**Baseline***	**End of EBRT**	**FU** (M12, M36, M60)	**FU** (M24, M48, M120)
IGF1	m	m	m	o
GH	m	m	m	o
ACTH	o	o	o	o
LH	m	m	m	o
FSH	m	m	m	o
TSH	m	m	m	o
fT3	m	m	m	o
fT4	m	m	m	o
Estrogen	m	m	m	o
Testosterone	m	m	m	o
Progesteron	m	m	m	o

Level of importance: m = mandatory, o = optional; *Baseline: At EBRT1 planning, or 2 weeks before or 1 week after EBRT1 1st fraction)

d. Sample Processing

1. Search for the puncture site:

The sleeve is rolled up over the elbow, whereby the rolled-up sleeve must not cause any stasis. Pay attention to the stretched but relaxed position of the arm on the arm pad. Apply the tourniquet 7.5 - 10 cm above the venipuncture site. Palpate and follow the course of the veins several times with the forefinger. Blocked veins lack elasticity roll very easily. If the superficial veins are hardly visible, then you can ask the subject to make a fist. As a rule, however, fist closure should be avoided, especially repeated fist closures ('pumping'). Slight tapping of the vein with the forefinger/ middle finger several times causes a swelling of the vein. Lowering the arm causes the veins fill up more and more. In difficult exceptional cases, damp heat can also promote the emergence of the veins: Hold a towel under warm water and place it on the crook of the arm for several minutes. Heat application belongs to the control deviations, which shall be documented in the patient record!

As soon as you have found a suitable site for the puncture (but no later than after 1 minute), ease the tourniquet!

2. Cleaning and disinfection of the puncture site:

Spray the puncture site with the skin disinfection solution of your choice and let it take effect for 30 seconds. Optional: Clean the skin at the puncture site by rubbing once with a cellulose swab. Wait until the place is dry, in order to avoid possible hemolysis of the blood by the disinfectant which leads to changes in various blood values. If the venipuncture appears difficult, you may have to re-puncture the vein. In this case, the affected area must be treated again with disinfection.

3. Puncture:

Tighten the skin of the subject by pulling distally with the thumb alone or with thumb and fore-finger. The wing cannula is kept in a position with the needle opening facing upwards. Use your eye to fix the area of the vein in which the needle tip should end. Then place the needle tip on the skin approximately half a needle length distally of this vein area. Then, with the needle in a vertical position, push through the skin with a gentle movement and then push the needle, which is now held flat, smoothly and evenly under the skin into the vein.

If blood flows into the tube, open the tourniquet immediately!

Place the serum (gel) Monovette on the Multi-Adapter, lock it by turning it clockwise and aspirate some blood as a sample. Should there be no blood flow, this usually means that the vein has been punctured. Pulling back the needle is no solution, because the vein wall is injured in two places and aspirating para-venous blood is strictly forbidden. This means that, in most cases, the needle must be inserted again, distally on the same or the other arm.

4. Filling the blood tubes (Monovettes):

Follow the filling sequence (first tubes without additives).

5. Remove the needle:

Place a cellulose swab over the venipuncture site. Remove the needle quickly then immediately afterwards (i.e. not yet while pulling it out) exert pressure with your thumb on the puncture site.

Ask the patient to apply the swab firmly to the puncture site for a few minutes. The arm must not be bent at the elbow. Make sure that the bleeding has stopped and then cover the puncture site with a plaster.

If a haematoma develops at the puncture site or the patient complains of pain, a bandage can provide relief. Immediately throw the wing cannula into the container provided.

e. Central Venous Catheter Blood Draws - Discard

a) Required supplies

Amount	Supply
1-2	Non-sterile gloves
2	Packet of sterile compresses
1	If necessary Syringe 5 ml
2	10 ml PosiFlush XS syringe
1	Octenisept Spray
2	IN plugs
1	5 ml ampoule of Medusana heparin 100 IU/ml (prepare 2x 2,5 ml)
1	Vomit bowl (for single use) as disposal

b) Performance - step by step

1. Disinfection of the work surface

2. Set up materials (see material list)

3. Hand disinfection

4. Open sterile compresses

5. Open 5 ml syringe package (for blood collection)

6. Prepare 2x 2.5 ml syringe with Medusanal Heparin

7. Prepare 10 ml syringes PosiFlush XS

8. Thoroughly spray a pack of compresses with Octenisept

9. Inform and position the patient

10. Put on non-sterile gloves

11. Remove protective compresses

12. Dispose of gloves

13. Hand disinfection

14. Put on non-sterile gloves

15. Place sterile compress under the Broviac

Note: Make sure that the terminals are closed.

16. Remove IN plug

17. Disinfection/cleaning of the connections with Octenisept compress

18. Change of gloves

Note: The terminal must always be closed, the stopper must be in place or the syringe must be connected.

For all following steps only use light pressure.

19. Carefully aspirate and discard 5 ml of blood at the thigh

20. Blood sampling

21. Rinse the Broviac with PosiFlush 10 ml XS

22. Block with 2.5 ml Medusanal Heparin 100 IU/ml

23. Close leg with new IN plug.

Note: Make sure that the clamps are closed.

(If necessary, repeat this procedure for the second Broviac leg.)

24. Repack the leg with sterile compresses, if necessary fix with plaster/crocodile clip

25. Dispose of material

26. Documentation

II. Anthropometry

a. Time Frame of Measurements

	Time points			
Parameter	**Baseline***	**End of EBRT**	**FU** (M12, M36, M60)	**FU** (M24, M48, M120)
Current sitting height (cm)	m	------	m	o
Current abdominal girth (cm)	m	------	m	o
Current hip size (cm)	m	------	m	o

Level of importance: m = mandatory, o = optional; *Baseline: At EBRT1 planning, or 2 weeks before or 1 week after EBRT1 1st fraction

References

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