Site visit report

Italy, Brescia
24.-25.5.2011

EHES
Measuring the Health of Europeans

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Site visit details

The site visit was conducted on 24th – 25th May 2011 at Brescia, Italy. This is one (the 12th) of the 20 Centres of the OEC/HES study, the Italian national HES. OEC/HES is conducted within the CUORE project – Epidemiology and Prevention of cardio-cerebrovascular disease (www.cuore.it). It has one Centre in each Italian region, except the two largest regions, which have two Centres each. The OEC is coordinated by the Istituto Superiore di Sanita (ISS, the Italian National Public Health Institute), in collaboration with the National Association of Hospital Cardiologists and partially supported by the Centre for Disease Control of the Ministry of Health. The fist OEC/HES was carried out in 1998 - 2002. The ongoing second study started in the first region in September 2008, and the fieldwork in all 20 centres is expected to be finalised by the end of year 2012. The target group for the study are all residents aged 35-79 years in the selected centres. A total of 9020 persons are expected to be studied. The fieldwork is carried out by local personnel from the Centres. The fieldwork in Brescia started in May, and will end early July.

The site visit was conducted by Kari Kuulasmaa and Päivikki Koponen of the EHES Reference Centre (EHES RC). The following persons were met:

- Simona Giampaoli, OEC/HES Leader/coordinator, ISS Rome;
- Pompilio Faggiano, assistant professor, preventive cardiologists, local leader of the HES in Brescia;
- Fieldwork team members:
    - Olga Cinelli, team leader, nurse;
    - Silvia Pezzetta, nurse;
    - Gloria Belleri, nurse;
    - Marytess Arcena, nurse;
    - Elisa Locatore, medical student;
    - Daniela Lonati, nurse;
    - Sara Vitali, nurse;
    - Paolo Martino, nurse (not met)
- Silvana Modoneri, Head of the biochemistry laboratory in the hospital of Brescia;
- Dr Ovidio Brignoli, Chair of the Association of GPs in Italy

Other key personnel of the OEC/HES coordinating centre at ISS, Rome, not met during this visit, are Francesco Dima, Chiara Donfrancesco, Cinzia Lo Noce, Luigi Palmieri and Serena Vannucchi. The key persons at the Italian National EHES laboratory at Cambobasso are Amalia deCurtis and Licia Iacoviello.

EHES RC reference samples were delivered to ISS earlier, on 27-28 April. The samples were stored at -80 for being analyzed within a few weeks.

Observations on the field work

In OEC/HES the fieldwork is organized in a fixed examination clinic in each centre. The fieldwork is carried out by local personnel. The number of persons and the timing of the examinations vary according to what is feasible at each centre. If the whole day can be used, the aim is to examine 20 persons a day. Some Saturdays and Sundays are also used. In Brescia, the examinations were carried out in the cardiology unit of the regional university hospital. The rooms were used for cardiologists' private practice in the afternoons, and therefore were available for the HES only in the mornings, when a maximum of 14 persons are examined. The sampled persons are instructed to show up at the examination site between 7:30 and 8:30, after 12 hour fasting.

In the first site visit day 12 persons were examined. Blood pressure was measured from all, followed by blood sampling. Thereafter the participants could have a self-paid breakfast in a near by cafeteria. After blood pressure had been measured and blood samples taken from all, usually by 10:00 am, the other examinations were started. The participants could leave the examination between 10:30 and 12:00. We both had the opportunity to follow and observe one participant each throughout their visit.

In the second day we focused on the information given to the participants, BP measurements and handling and processing of the laboratory samples.

Field work site

The participants were examined in six points. The participants were invited to the rooms in the order of the number they were given at entry in the morning. Sometimes there seemed to be slight confusion as to who goes next to which room, but these were settled quickly by the team leader or OEC/HES leader.

The examination visit includes:

- a "lesson", explaining the survey and the informed consent, for all participants of the day in the morning. invited for one day (explaining the survey and consent issues in the corridor/waiting room).
- a personal appointment for registration and signing the consent, instructions for the self-administered food questionnaire (from EPIC) and the health module MEHM-ADL-IADL questionnaire (measurement point 1),
- measurement of BP and heart rate (point 2),
- blood sampling (point 3),
- breakfast,
- interview (CAPI) (point 4 or 5),
- ECG, anthropometric measurements and bone densitometry (point 4 or 5),
- spirometry, checking the self-administered food questionnaire and last giving a container and instructions for the 24 h urine (point 6).

The results are given to the participants when they come back the other day to bring their urine samples. Information on health promoting lifestyles and purpose of the measurements is given in the folder in which they receive their own results. The examination visit took about 4 hours per participant, with 15 minutes-1 hour waiting times between the measurement points.

The order of the measurements may vary by region, but blood pressure is always measured first. Carbon monoxide measurements are also carried out in some regions, but not in Brescia. Some other measurements (Bone densitometry and complete hemochrome (blood cell count, haemoglobin, hematocrit, etc) may also be included, if the region agrees (most regions have agreed), but the EHES core measurements and ECG are done in all regions.

Composition and collaboration of the fieldwork team

The composition of the fieldwork team may also vary by region. Mostly the measurements are carried out by nurses who work full-time with the OEC/HES. The nurses in Brescia are working part-time with the project, part-time in their normal hospital work. Each day, the examinations were carried out by four nurses, one of them a leader of the team, and a medical student carrying out the interviews. Altogether, seven nurses had been trained and approved by ISS coordinators/trainers. They rotate between the days. The nurses may also rotate their tasks and carry out different measurements at different days. They rotate their tasks even during one day as the nurses first work in three –four rooms to take care of the reception and consents, measure blood pressure and take blood samples, then they continue with the other measurements.

Reception and obtaining informed consent

The lesson given to the participants can be repeated several times when participants arrive. The purpose is to share key information on the purpose of the survey to several people at the same time, so this does not have to be repeated individually to each participant. When the OEC/HES leader is present she gives this lecture herself. When she is not present it is the task of the team leader nurse. The lecture takes about 5 minutes, pointing out the key issues. The most common question the participants ask is "Why I was selected, can you take a member of my family who needs the health examination" and the usual answer is "This is a random selection, sorry we cannot take others":

Numbered envelopes with all papers, recording forms and the dietary questionnaire for the participants have been prepared. At the reception (i.e. first examination point), the participant received the next number and got the envelope which had this number. All forms in the envelope had this same number. On the top of the first page of the recording forms there was a log where each examination point was ticked, when completed.

The consent is given separately to:

- voluntary participation in the study,
- permission for storing blood in the biobank, and
- permission for use of personal data.

Three copies of the consent form are signed: one for ISS, one for the participant and one for the region/local hospital. There was discussion between the nurse obtaining the consent and the participant before signatures. Most of the information had been given to the participants earlier, in the invitation letter and information notice mailed to them, and in the lection on arrival to the examination site.

The first nurse obtained the consent, checked the participant's identity from her personal identity card, checked her contact information, asked self-reported height and weight, and gave the food questionnaire, and instructed the participant to wait for invitation to the next room. She also gave instructions to complete the food questionnaire while waiting

In the hallway, which served as the waiting room, there were usually 8-11 participants waiting for invitations to the examination rooms. They were filling in the food questionnaires and talking with each other. The long waiting times did not seem to bother them.

Measurement procedures

Blood pressure and pulse

At the second point, the nurse measured the arm circumference, asked about fasting time and a few other short questions, and recorded the room temperature before the blood pressure measurement.

Device and protocol: The mercury sphygmomanometer is used. Three measurements are taken after 5 min rest and one minute between the three measurements. The pulse rate is measured for one minute at the end of the first BP measurement. Two cuff sizes are available (normal adult, bladder size 11.5x23cm, and obese, bladder size 12x40cm). These are in agreement with the EHES recommendations, except that a small cuff was not available.

Observations: The measurements were carried out following the EHES protocol and the position of the subjects was correct. As an extra, the tubes of the BP device were taken apart before and between each measurement. This was explained to be done to assure the zero level of the mercury. The resting time before measurements and between measurements were not timed, and were perhaps a little shorter than instructed. The window of the room was open and there was noise from the traffic, which could disturb the auscultation. Regularity of the pulse was not recorded. It was thought that this is too difficult for the nurse to judge, and this can be seen from the ECG when needed.

After the BP measurement, the participant was instructed to wait for invitation to blood sampling.

Blood sampling

At the third point, six tubes of blood (three for serum and three for EDTA plasma) were taken to determine total and HDL cholesterol, triglycerides, fasting glucose and complete hemochrome (to be examined in the local laboratory). The number of tubes may vary, depending if there is interest in the region to include hemochrome and other potential analysis. Fluoride EDTA tubes are used for glucose measurement.

Observations: a rubber band was used as a tourniquet and it was kept tightened throughout the blood drawing. However, the blood drawing was fast. There were two nurses present, one taking the samples and the other one giving the tubes and fixing the labels. The participant's number, name and date of birth was hand written in the labels. Bar code labels are fixed later in the laboratory, when samples are processed for freezing and transfer to the national laboratories.

After the blood samples were taken the participant was instructed to go and have breakfast (at their own cost) and to return for further measurements.

After the participants returned, most of them had to wait more than one hour, as the other measurements were started only after all blood samples had been taken.

The blood samples were collected between 8 and 10 o'clock. After all samples were taken and the urine samples brought by the earlier days' participants had been handled, they were taken to the hospital laboratory. Thus the time between blood drawing and centrifuging varied between 1-3 hours, sometimes even 4 hours.

Interview

The interview was carried out in one room (point 4 or 5 for the participant).

The computer assisted interview covers the EHES questionnaire (except the self reported height and weight, which were asked earlier). Additional items cover smoking (from EHRM), physical activity (Italian validated questionnaire), alcohol intake, salt intake, questions for women (menopause) and the use of health services. In addition the Mini-mental state examination (MMSE) is included for persons aged 65 and above, but we did not have a chance to observe this.

The interviews lasted about 15-20 minutes. The interview started by entering the personal information (asked in the first room) and blood pressure results. Other measurement results are entered to the same file at the end of the day after all measurements have been taken and all participants have been seen.

Observations: The interviews were carried out correctly and efficiently. The questions seemed to be asked verbatim and the interview proceeded smoothly. The interviewer had good eye contact and communication skills to build a friendly atmosphere for the interview.

After the interview, the participant was instructed to wait in the hall for an invitation to the next room.

ECG, anthropometric measurements and bone density

In one room (point 4 or 5 for the participant) two nurses carry out the measurements. The participant is instructed to undress and the measurements are taken in light underwear.

First the 6 lead ECG is taken at rest with the Mortara E230 device. Two copies are printed: one for the participant and one for the study to be later coded at ISS by a certified reader according to the Minnesota code (9 items).

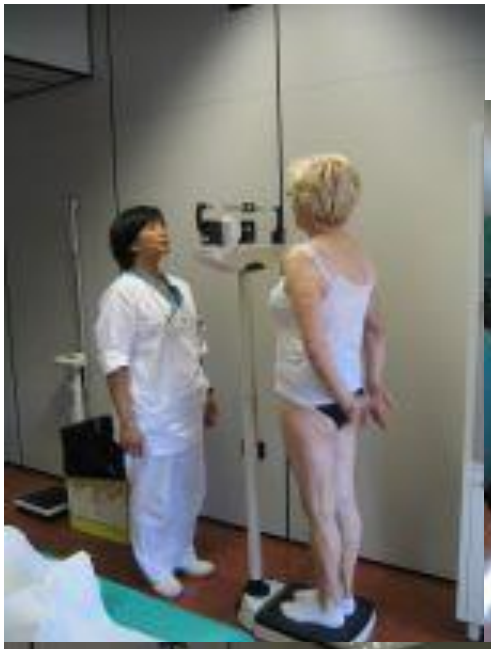
Weight is measured using the balanced beam scale (Seca model 711), height with a height rule taped vertically on the wall surface and a carpenter's try square.

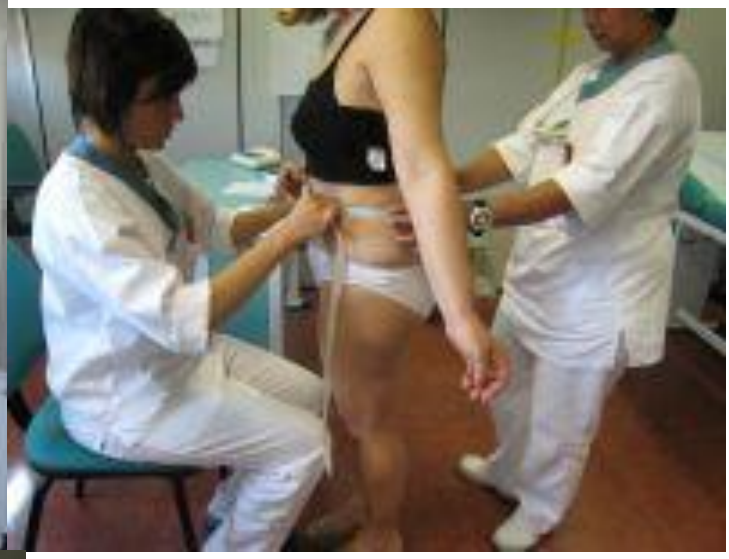
Waist and hip are measured with a non elastic measurement tape with the measurer sitting and the participant standing straight with feet a little apart.

After dressing, the bone density is measured from the heel by the Achilles InSight device.

Observations: The two nurses carried out the measurements, with one taking the measurements and the other one assisting and recording. For the measurement of waist circumference, this allowed a quick checking of the placement of the measurement tape in the back. However the placement of the measurement tape was not assessed by feeling the lower rib margin and the iliac crest. The national instructions specified waist to be measured in the midway way between the lower rib margin and the iliac crest.

After these measurements the participant was instructed to wait to be seen in the last room.





Spirometry, instructions for the urine collection and checking the questionnaires

In the last room (point 6 for the participant), spirometry was taken with a portable device SpiroPro. FCV and FEV1 are assessed. The results are later printed and recorded. The participant receives a printed curve with the respiratory volume values. First the technique was instructed and then two blows were made. The device records the best values.

In addition to the interview questions there is a self-administered dietary questionnaire from EPIC. Most participants were able to fill it in while waiting for the measurements. It was checked in the hall while waiting or in the last room. The checking aimed to ensure completeness and clear markings to allow data entry by scanning. The MEHM questionnaire and ADL/IADL items (from ISTAT) are also checked for all participants.

At the end, the participants are given a container (with timol as preserver) and instructed for the collection of 24 hour urine. It is collected for the determination of urinary sodium, potassium, creatinine, microalbumin and iodine secretion. When the participant brings the sample in the morning, the volume is measured, and four 10 ml bar-coded tubes are filled in. There are tubes for the above analyses in three different university laboratories (Campobasso for microalbumine, Pisa for iodine, Naples for sodium and potassium), and one tube for being stored at -80°C at ISS, Rome.

Carbon monoxide measurement

In some regions the Carbon monoxide measurements are made using the Micro Smokerlyzer. This was not done in Brescia.

Deviations from the EHES protocols

Almost everything was done according to the EHES protocols. More attention needs to be given to placement of the tape for waist measurement and quietness of the room during BP measurements. Some informal discussion with the participant before BP measurements could help him/her to feel more relaxed.

Feedback to the participants

The local cardiologist reviews the results of all participants after the measurements are finalised for the day. He checks especially the ECG, to give feedback to the participants when they come back with the urine samples. If anything abnormal is observed in the ECG or other measurements or lab values, the participant receives a call from the doctor to check that they are already under control. Nearly all have already been aware of the identified medical conditions.

When bringing the urine sample, the participant receives a folder with:

- information on the measurements and health promoting lifestyles,
- the participant's copy of the consent,
- results/prints from ECG, spirometry and bone densitometry,
- an individual risk score or a risk chart printed from the data entry programme,
- laboratory results (hemochrome, total and HDL cholesterol from the local laboratory) .

The participants receive no feedback about the urine analysis.

Getting the personal results seemed to be a key factor motivating participation. In addition to receiving personal results and personal advice, it is pointed out for the participants that this study is important to develop research for prevention; the next generations will benefit of these research.

The feedback discussion is brief, and the participants are instructed to show their results to their own GP. The feedback was given in the waiting area (hall) or more privately in the room if there was one available. During our observation days, the OEC/HES leader gave the feedback. When she is not at the site, the feedback is given by the team leader nurse.

Discussions with survey participants

Because of lack of a common language we were unable to discuss with the participants.

Discussion with the coordinator/leader

Sampling and recruitment

The sample is taken from the list of residents, with information on name, date of birth, sex and address, taken from the list of residents of the municipality. The quality of the list (when it is updated) may vary by region/municipality. The sample is stratified by age and sex. ISS has been in contact with Johan Heldal to discuss the sampling issues, to evaluate the sampling scheme of the ongoing survey and to develop procedures for future surveys. The sample is larger than initially invited, and in cases of non-response, replacement is made from the sample. This replacement procedure does not affect the non-response bias, but may complicate the weighting of the persons in the data analysis. However, a record of the invitation and response status of each person in the sample is kept, and it will be possible to exclude the replacements from data analysis.

The sampling procedure need to be re-thought carefully for the next national HES. Ideally the sample would be selected in such a way that both (a) the survey would be representative of the whole country (instead of the currently selected municipalities) and (b) trends from the current survey could be assessed.

Recruitment process:

- An invitation letter is mailed with an appointment time and the information notice. In Brescia the letter is signed by the OEC/HES leader on behalf of the CUORE project and ISS, and the chief medical doctor of the regional cardiology unit. The persons are instructed to call in the mornings and confirm the appointment but sometimes they come without confirming. In the letter, the sampled persons are also encouraged to call if they have any questions. The team leader nurse answers to these phone calls.
- A second letter is mailed if the person does not show up to the visit, after about one week, with a new appointment.
- If the person does not show up to the second appointment a phone contact is attempted. These phone calls are made by the local staff, and they may make different efforts to reach the person. It seems that people do not like to receive the phone calls because they are annoyed by frequent marketing calls. It is not common that people participate after the phone call.

The contents of the invitation letter may vary with regional interests (e.g. differences in who signs the invitation).

No non-respondent questionnaires are used. This is considered not feasible, as the people do not seem willing to be disturbed with phone interviews. The vital status of participants and non-participants has been checked. It was discussed that a short mailed non-respondent questionnaire could be piloted in the pilot areas later.

Ethical and data protection issues

The Ethics Committee of the ISS examined all documents related to the OEC/HES and approved all aspects of the study before it was started in 2008. Later participation in EHES was approved by this committee in 2009. The regions may decide if presentation to regional ethics committees is needed. This is only to inform about the study, as the ISS committee decisions are considered to cover the data collection in the whole country. The concerns raised by the Ethics Committee were:

- Why it is important to include also fragile elderly people (e.g. with dementia)?

- What is given to the participants? It was needed to point out that they receive only suggestions for lifestyles, not medical care, to avoid competition with what the GPs are doing.

No concerns in data protection were observed, except that occasionally personal health issues or feedback to the participant were discussed in the corridor/waiting room, where also other survey participants were present.

Selection of the survey site

The OEC/HES is carried out in one municipality (Centre) in each Italian Region, except in the largest regions (Lombardia, Veneto and Piemonte), where two Centres are selected. The centres are selected non-randomly, based on the willingness of the hospitals/regional cardiology units to take part in the study. The centres also need to organize the rooms for the examination site, and to provide the field-work personnel, who will be paid centrally by the national study. The centre will also need to organize the facilities for blood sample processing and analysis of the results which will be given to the participants.

We got the impression that the examination site can be reached easily by all residents of the municipality, although this was not discussed explicitly.

Selection of the field work staff

The nurses are selected at local level from the staff at the hospitals and their participation is approved after their interest and commitment is evaluated by the OEC/HES leader.

Training of the field work staff

The local staff is trained by the OEC/HES coordinators during the first week(s) of the data collection. In the first days the trainees observe the example of the trainers. Then they start to carry out the measurements and get feedback from the trainers. The double stethoscope is used to check the readings of blood pressure measurements. Differences more than 2 mmHg between the trainer and the nurse are not allowed.

The survey coordinator feels that it has been challenging to train the nurses to see the healthy people and understand the aim of keeping people healthy. It is emphasized that this is made for health, not diseases. The nurses in Italy are not used to independent practice and no public health nurses are available.

Data management

A system (CuoreOEC) for computer assisted interview and data entry had been prepared for the survey by an outsourced software company. The same system is used to enter the paper forms of the measurement results and blood results from the local laboratory. At the end of each section the software alerts users of missing or incomplete data before saving the information. The electronic questionnaire allows a first assessment of the quality of answers as no inconsistencies are allowed during data entry.

The order of the data items in the blood pressure recording form was different from the order in which they were recorded. A good justification for this was that it now corresponded to the order in the data entry programme. If the latter will sometimes be updated, it would be good make the order consistent with the measurement procedures.

The system calculates a CVD risk score, derived from the earlier prospective follow-up of the CUORE project. It also prints the results to be given to the participant when he/she returns the 24-hour urine sample.

The self-administered EPIC food questionnaire is read by an optical scanner. This service is bought from the Italian Cancer Institute. ISS receives the raw food data as well as the nutrient data derived using the food composition tables of the Cancer Institute.

The data are stored locally in the laptops and backup files are taken to memory sticks, usually less frequently than daily. There have been no accidental data loss so far, but it is strongly recommended that a routine back-up is taken every day, and preferably several times a day. The OEC/HES leader transfers the samples and data in memory sticks from the Centre to ISS.

Quality assurance procedures

Data checking in the data entry programmes as described above. No other data based quality control is done in the Centre, but at ISS after the fieldwork period of each centre. Frequencies and last digit preference in BP values are checked, but not individually by the measurers. A wish was expressed that the EHES RC could provide software for this for use in countries.

The trainers/coordinator observe the measurements, mainly during training, but also later when they visit the fieldwork sites.

Visit to the local laboratory and the ISS Biobank in Rome

The sample processing and laboratory analysis may vary by region, based on what is feasible at each site.

At the end of the day (in Brescia between 10 and 11 am), the team leader nurse takes the blood and urine samples to the hospital laboratory. One blood tube/ participant is left for the complete hemochrome analysis. The results of this analysis are sent to the preventive cardiologist of the hospital who reviews the results.

The four urine samples and the remaining 5 blood tubes/participant are taken to another laboratory unit. The urine samples are frozen at -20°C for transfer to the relevant laboratories. The blood samples are centrifuged immediately for 10 minutes. The centrifuging speed was 3200 rpm. For the recommended relative centrifugal force of 2000g this requires a radius of 17 cm from the tip of tube to center of rotor. We did not measure the radius, but it seemed that it was much less. The Centre should check this, and increase the centrifuging speed if needed.

The samples are analyzed in the laboratory in the same day and the results are provided to the participant from the next day. All laboratory analyses for the national health monitoring are conducted in the national HES laboratory in Campobasso. Samples for this are frozen at -20°C. The rest of the samples are aliquoted according to the EPIC protocol with a specific device into straw tubes for being stored in the biobank at ISS, Rome. Also these are stored at -20°C, transfer at -80°C during the screening period and in liquid nitrogen after transfer to Rome. The transfer to Rome is in dry ice

The straw tubes are labelled with bar codes, the bar codes are also attached to the participant list and to a list with information on the number of serum, plasma and buffy coat tubes per each participant. The type of samples is marked with colours in the tubes. The sample processing was well organized and the head of the laboratory, Silvana Modoneri has written a detailed description of the sample processing.

The frozen samples are transferred to the respective laboratories at the end of the fieldwork in the centre.

Two paillettes of serum preserved at -80°C are sent to Campobasso for the centralised tests of total and HDL cholesterol and fasting blood glucose (EHES measurements); 4 types of straws at -196°C in liquid

nitrogen are preserved in the biobank of the CNESPS-ISS marked with different colours: 4 of serum (yellow), 6 of plasma (red), 2 of buffy coat (blue), 2 of packed red cells(green).

During the earlier meeting in Rome we had a possibility to visit the biobank at ISS. We saw a well organized biobank system (tanks of samples in liquid nitrogen), developed for several years at ISS and now functioning well with an alarm system and a well organized sample management system.

According to the OEC/HES leader, glucose was measured in Campobasso from serum. This is problematic due to the fact that the samples were centrifuged 1-4 hours after drawing, and the glucose is burned by the cells all this time. EHES recommends the use of fluoride and citrate in the samples to keep the glucose concentration stable. There was a plan to test the use of serum, EDTA plasma and fluoride citrate plasma simultaneously in the forthcoming survey site in Torino.

Feedback discussion between EHES RC and local team

We gave short feedback to the nurses and the interviewer at the end of the first day. We thanked the staff for the good work and well done measurements as well as keeping the participants satisfied despite the long waiting periods. More attention should be given to the waist circumference measurements and resting time before and between blood pressure measurements, as well as on quietness of the room during blood pressure measurement.

The nurses felt that they had received good training. They appreciated the possibility to learn new things and to get experience from a different type of practice than their normal work. They feel that they learn a lot and have good collaboration with the ISS staff.

Meeting with other key persons

We met Pompilio Faggiano, assistant professor of preventive cardiology of the regional cardiology unit who had helped with organizing the OEC/HES in Brescia. The need for developing preventive cardiology and the use of survey data was discussed. We also met a local GP, Dr Ovidio Brignoli, who is the chair of the Association of GPs in Italy. GPs were considered to be key persons to encourage participation among their patients and to interpret the personal results of their patients. They also need the survey results to develop preventive care in their practice. Collaboration of the survey leader Simona Giampaoli and the cardiologists

and GPs seems to be one of the corner stones of OEC/HES. In Italy, the OEC/HES is unique, and no other national population health surveys exist. ISS is also organizing a HES for children (OKKIO study) led by Angela Spinelli, where children are recruited from schools.

Conclusions and recommendations

The Italian OEC/HES has been built since the 1990s. It is based on collaboration with regional authorities and the methods have been adapted to what is feasible in each region. A specific characteristic of the Italian survey is that separate local fieldwork teams are recruited for each centre (region). This is challenging for the training. Therefore, particular attention during the site visit was paid to the training procedures and the performance of the fieldwork staff. Our observations were positive, and the team was very dedicated to the work. Future surveys can no doubt be built on this excellent work.

The main challenge in Italy is the participation. The impression was that rural areas are easier for recruitment and largest cities most challenging, but not much can be done to motivate people to show up. Perhaps one solution could be to increase the awareness of the population about the survey, so they will recognize it when they receive the invitation. So far the participation rates have varied in the Centres from 40 to 78 %.

As always, it is possible to further enhance standardization and quality control. Specific issues on this are summarized below:

- More attention should be given to the position of the measuring tape for waist circumference measurements;
- Attention should be paid to sufficient resting time before and between blood pressure measurements.
- Quietness of the room during blood pressure measurement should be ensured. During the site visit the window was open and noise from the traffic could disturb the auscultation.

- We did not get a good record of the tubes used in blood drawing; for the centralised blood measures and preservation in the CNESPS-ISS biobank 1 blood collection tube of serum of 10cc and 2 blood collection tubes of 4.5 cc with EDTA are recommended; these quantity allow to preserve 6 paillettes of serum (2 sent to Campobasso for the centralised tests) 6 of plasma, 2 of buffy coat and 2 of packed red cells; for local blood analysis (to be given to the participant) 1 tube of serum and 1 tube of 4.5cc with EDTA. All the blood collection kit is available at local level and differs from centre to centre.

- It is strongly recommended that a routine back-up of the data entry system is taken several times each day.

- The relative centrifugal force should be calculated using the formula in the EHES manual. If it is less than 2000g, the centrifuging speed should be increased.