

MINI-SYMPOSIUM: COHORT STUDIES

How should a birth cohort study be organised? Experience from the German MAS cohort study

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KEYWORDS

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Summary Birth cohort studies offer the opportunity to study average risks, rates and occurrence times of disease longitudinally from birth. The effect of genetic and environmental factors and their interactions can be studied. Furthermore, quantity and duration of exposure to environmental agents can be evaluated prospectively.

However, prospective birth cohort studies are expensive, labour intensive and take many years to complete. Loss of subjects over time as well as recall bias complicate the interpretation of observations. This paper summarises the potential pitfalls of such studies and discusses the experience of the German Multicentre Allergy Study (MAS), which began in 1990 in five German cities and included 1314 newborns for the study of the natural course of atopic diseases.

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INTRODUCTION

In epidemiology, the word *cohort* describes a group of people who share a common experience or condition. For example, members of a birth cohort share the same year or period of birth.

In the hierarchy of evidence, prospective cohort studies are considered to be more valid than cross-sectional or case-control studies. Epidemiological cross-sectional studies often reveal potential associations between disease rates and certain exposure factors. However, associations found in cross-sectional studies have to be interpreted cautiously with respect to causation and outcome variables. The prospective, longitudinal observation of a birth cohort allows the evaluation of exposure to environmental agents from early life and temporary effects can be studied as well as consistent effects. However, to prove a causal relationship between exposure and disease, intervention studies are required for all types of epidemiological studies.¹

The first symptoms of atopic disease are often observed in infancy. Therefore, prospective birth cohort studies have

been conducted to prospectively study the natural course of atopy-related traits and to identify early risk factors and predictors.^{2–7}

The German MAS cohort ($n = 1314$) was recruited in 1990 and followed to the age of 10 years. Study design (outlined in Fig. 1), selection and recruitment of families, diagnostic procedures (shown in Fig. 2), analytical approaches as well as measures applied to prevent loss of subjects will be described.

PLANNING A BIRTH COHORT STUDY

Planning of a birth cohort study requires a great deal of time, money and interdisciplinary teamwork between epidemiologists, biostatisticians, data managers, gynaecologists and paediatricians.

Study centres

Large obstetric departments located near future follow-up visits are recommended for the recruitment of families. Multicentre studies within one country are recommended

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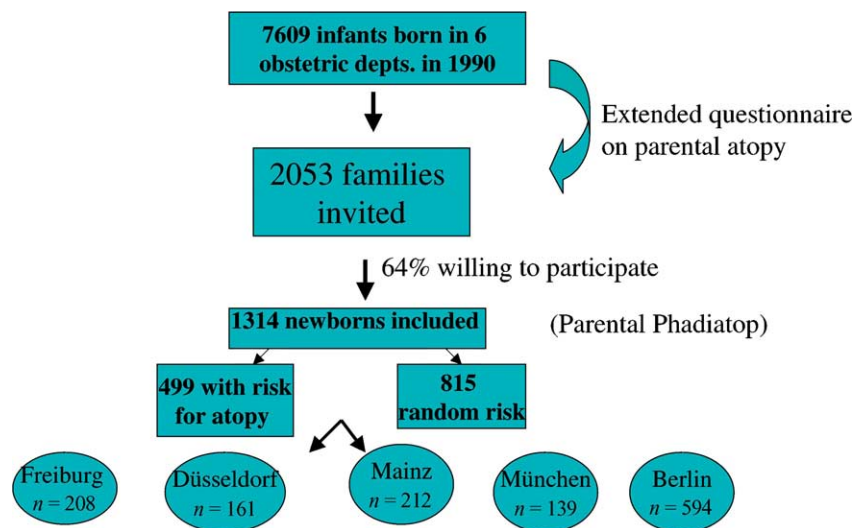


Figure 1 Study design and selection of newborns in the German Multicentre Allergy Study (MAS).

not only to increase the numbers of the cohort but to provide the opportunity for evaluating the role of living conditions, differences in exposure to environmental agents and their effect on disease prevalence in different cities. For

example, in the MAS study, we observed highly significant differences in house dust mite allergen (mite group 1) concentrations in carpet and mattress dust samples, with eightfold higher values in Freiburg, a small city in Southern Germany compared to Berlin and Munich (Fig. 3).⁸

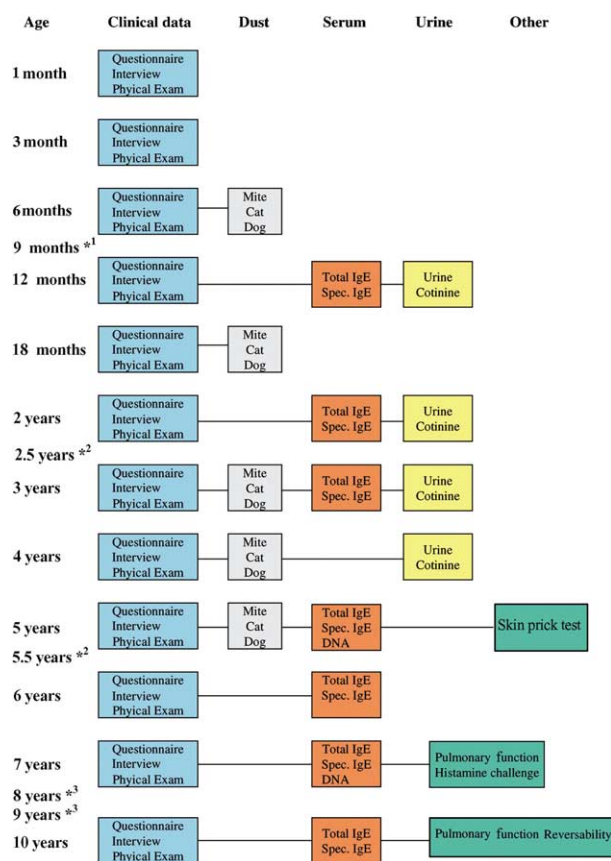


Figure 2 Follow-up visits from birth to the age of 10 years and diagnostic procedures at respective timepoints in the MAS. *Questionnaire data only, children did not come to the study centre; ¹repeat questionnaire; ²psychosocial questionnaire; ³ISSAC-based questionnaire; Spec. IgE, specific IgE.

Personnel

It is pivotal to have enough experienced personnel (physicians, nurses, statisticians and technicians) who will stay as long as possible throughout the study. Changes in personnel (particularly of study nurses) usually induce a high drop out rate. In our experience, study nurses should be employed full-time for a birth cohort study, their responsibilities include arranging follow-up visits, making telephone interviews, undertaking clinical duties at follow-up visits (assistance with blood sampling and pulmonary function testing, measuring and weighing the children and collecting the questionnaires and biological samples). Parents of cohort-study participants should be able to contact the study nurse when they have questions about their child's health or the results from allergy tests. Study nurses should be present at follow-up visits since children and parents feel more comfortable with procedures when a familiar person is present.

Size of study population

Biostatisticians should propose the number of infants that it is necessary to include taking into consideration a minimum yearly 10% drop-out rate. The longer the observation period the greater the number of children required. To obtain stable estimates of incidence requires a substantial number of patients with the disease. If a rare disease is being investigated it is wise to increase the number of infants at risk for that specific disease, by selecting parents who are affected. However, if the results are to refer to the population-based risk, it is necessary to recruit a population-based

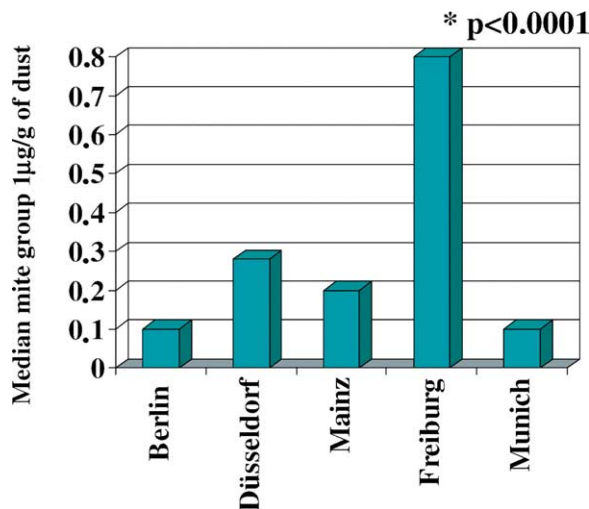


Figure 3 House dust mite allergen exposure (Der p 1 + Der f 1 µg/g of dust) in carpet dust in five German cities.⁸ Number of samples = 939.

cohort; otherwise the results will need to be mathematically corrected.

PRE-RECRUITMENT PERIOD

Recruitment of subjects should not start until logistic issues have been finalised:

- Detailed study protocols need to be developed. Midwives and obstetricians should be motivated to collaborate. A detailed, easy-to follow protocol for processing cord blood samples (if required) must be made familiar to all midwives. All materials (tubes, syringes, supplements) must be provided.
- Validated questionnaires/interviews must be available, preferably tested in pilot studies.
- Proband codes used for blinding, data entry, transfer and processing have to be clarified prior to the recruitment of subjects. Appropriate hard and software must be available. In the MAS, personal data (names, addresses, telephone numbers) were only accessible in the local study centres. Interviews, questionnaires, results from pulmonary function tests as well as serum samples obtained in external study centres were sent to Berlin labelled with 6-digit codes followed by the number of the follow-up visit. Data entry took place in Berlin.
- Labelling (stable upon freezing!), transport and storage of biological samples (particularly in multicentre studies) to a central laboratory must be meticulously organised. In the MAS, external centres collected frozen serum samples prior to shipping at 3-monthly intervals. Laboratory analyses and storage of remaining material (dust extracts, serum) should take place in one laboratory.

- Sufficient freezer space should be organised at the outset.
- If the study is not conducted in a paediatric unit, basic prerequisites (baby scale, measure tape, radiator etc.) as well as blood sampling tools have to be organised.
- The study protocol and written informed consent forms have to be approved by the ethics committee (all local ethics committees if multiple centres are involved). To avoid resubmission/amendments, the extent of the study should be planned in advance.
- To avoid future conflicts, investigators should outline their major interests and hypotheses at the commencement of the study.

SELECTION AND RECRUITMENT OF FAMILIES

During the recruitment period midwives and obstetricians should complete a screening questionnaire for women admitted in labour. A minimum of questions (e.g. MAS parental history of asthma, allergic rhinitis and/or atopic dermatitis) should allow the study physicians to select families for more detailed evaluations shortly after birth.

Multiple follow-up visits are required over a long period of time. Although this confers a potential bias, couples with first-born children will be more likely to keep scheduled appointments and carry out tasks (e.g. keeping a diary, collecting dust samples).

The vast majority of MAS children are of bilateral German ethnicity, mainly because communication in German with mothers in order to answer questions and fill in questionnaires was a prerequisite for the study. However, when planning a birth cohort study, particularly when inflammatory disorders are studied, ethnicity should be taken into consideration. Studies have demonstrated not only significant differences in the prevalence of atopic disease among different ethnic groups,⁹ but also in the frequencies of genetic variants of proinflammatory genes, which vary dramatically between Caucasian, Asian and African populations.¹⁰ Therefore, if cohort studies are not limited to one ethnic group, ethnicity of both parents should be documented to allow separate analyses of different ethnic groups.

Birth dates of newborns selected for a cohort study should ideally be evenly distributed over a 12 month period to allow for seasonal effects on outcome parameters.

If risk factors for a disease later in life are to be identified in a birth cohort, the study population should be restricted to healthy, mature singleton newborns.

RECRUITMENT AND VALIDATION OF INSTRUMENTS IN MAS

More newborns at risk for atopy were recruited than in a population-based study. The selection of newborns with an

increased risk of developing the disease is commonly performed to limit the overall number of subjects required to test hypotheses on disease onset and progression. However, when the natural course of biological parameters in a general paediatric population is to be estimated using data from a high-risk enriched cohort, the cohort has to be weighted by the risk factors on which the selection procedure for the respective study population was based.

Family history has a high predictive value at birth for later atopic disease.¹¹ Moreover, in 1984 an elevated cord-blood IgE of >0.9 kU/l was considered to be a valid predictor for later manifestation of atopic disease in infancy and childhood.¹² To screen newborns for a prospective cohort study about the aetiology and course of atopic diseases, we developed two family questionnaires to identify atopic family members. In the delivery room three questions concerning atopic manifestations (allergic asthma, allergic rhinitis, atopic dermatitis) were included in the general admission procedure.

A second, more detailed, questionnaire was developed by paediatric allergologists and epidemiologists. The aim was to screen parents and siblings of newborns for atopic diseases. The questionnaire asked for typical allergic symptoms after exposure, for atopic diagnoses, allergy tests and results, smoking status, education and occupation of the person who filled in the questionnaire. If at least one of these screening conditions turned out to be positive, the family member was considered to be atopic.

A total of 6019 (79%) mothers and 5819 (77%) fathers of 7609 infants born in six obstetric departments in five cities (Berlin, Munich, Mainz, Düsseldorf and Freiburg) between January and December 1990, filled in the questionnaire. Of these, 22% of the mothers and 21% of the fathers were classified as atopic; 64% of all families invited participated in the study. A total of 499 infants with a double-positive family history and/or cord blood-IgE values above 0.9 kU/l were recruited as cases (all infants at risk available) and 815 infants, selected at random from the remaining newborns, were recruited as infants at random risk (Fig. 1).

Furthermore, an internationally accepted screening test for specific IgE-antibodies to common inhalant allergens (Phadiatop, Pharmacia) was performed. Of the 1314 participating families, 793 mothers and 353 fathers were willing to have a Phadiatop screening test applied within the first 6 months. There was no significant difference in atopic status (by questionnaire) in the mother tested, compared to the mothers in the total cohort, i.e. 35% and 36%, respectively. The same was true for fathers: 30% were atopic in the total cohort and 27% of those tested.

THE FIRST YEAR

In the MAS, short intervals between the first follow-up visits (at the age of 3, 6, 12, 18 and 24 months) were scheduled (Fig. 2). This approach was not only chosen to detect subtle

signs and symptoms of atopy (particularly atopic dermatitis) and to minimise recall bias with regard to feeding patterns and infectious disease but also to form a stable relationship with parents. In addition, the study nurses and physicians could be contacted by telephone by parents with any concerns about their baby any time during working hours (messages left on answering machines at night-time or weekends were answered as quickly as possible). Parents appreciated the contact. However, the non-interventional character of a study may be at risk when parents seek advice for prevention of disease from experts in the field.

LOSS AND TRACING OF SUBJECTS

In principle, a cohort study could be used to estimate average risks, rates or occurrence times of disease. Except in certain situations, however, information on average risks and occurrence times of disease cannot be directly derived from the observation of a cohort. The description of average risks or time courses of specific events requires that the entire cohort remains at risk and under observation for the complete follow-up period. Loss of subjects during the study period will prevent direct measurements of these averages, since the outcome of lost subjects is unknown. Thus, the only situation in which it is feasible to measure average risks and occurrence times directly is in a cohort study in which there is little or no loss to follow-up. Follow-ups that trace less than 60% of subjects are generally regarded with scepticism, but even a follow-up of 70% or 80% or more can be too low to provide sufficient assurance against bias.

Birth cohort studies that span many years present logistical problems that can adversely affect validity. Tracing of families adds to the costs of prospective cohort studies, yet the increasing mobility of society warrants stronger efforts to trace subjects.

In the MAS, during the first study year the loss of subjects was 15% and 2.5–5% annually thereafter (Figs 4 & 5). High drop out rates were observed when

- A study centre was moved to a different location within a city (twice in Berlin),
- Changes in personal occurred.

The following measures appeared to be helpful in motivating families to continue to participate and in preventing loss of study participants:

- The address and phone number of the grandparents as well as work phone numbers was recorded. At each follow-up visit parents were asked whether they planned to move.
- Local anaesthetic skin lotions (EMLA[®]) were offered prior to blood sampling. To avoid prolonged waiting times EMLA[®]-plasters were mailed together with the invitation once children were 5 years old. Parents were instructed to apply plasters on a visible vein in

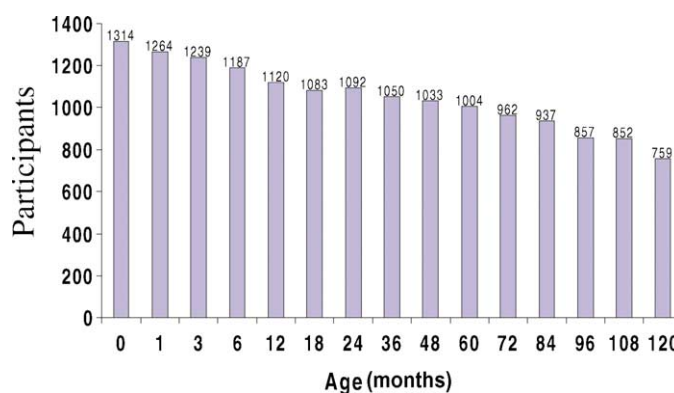


Figure 4 Participation rates from birth to the age of 10 years in the MAS, 1990–2000.

the cubital area or on the hand 30 minutes prior to the scheduled follow-up visit.

- Multiple phone calls were performed to schedule and confirm appointments. In addition a written invitation with the description of procedures to be performed was sent 1–2 weeks prior to the appointment. It did not prove to be useful to schedule appointments for the following visit a year in advance.
- When parents were unable to come to the study centre, home visits were offered.
- Telephone interviews were conducted when visits at the study centre or at home were not possible (e.g. because families moved).
- Free parking was available for families at follow-up visits.
- Parents/children were not paid for their participation; however, reimbursement for transportation costs was offered. Also, children received small gifts at the end of every visit.
- Results from allergy tests and house dust analyses were sent to the families.

- MAS-journals with descriptions of ongoing and planned studies (with photographs of investigators) as well as summaries of published results were sent to all MAS-families (e.g. together with Christmas greetings).
- In Berlin (the largest study centre) MAS parents' evenings were organised where developments in asthma and atopy research as well as results from the MAS study were presented.

These measures were taken irrespective of children's atopy status. In birth cohort studies participation bias may occur over time with onset of disease, i.e. affected families are more motivated to stay in the cohort. In the MAS, no severe bias could be observed although at 7 years, complete information was available in only 939/1314 individuals. Families who smoked were more likely not to participate at age 7 years.

EXPOSURE

Exposure can be acute or chronic. The definition of a chronic exposure is more complicated. We must imagine a period during which the exposure accumulates to a sufficient extent to trigger a step in the process.

Another problem is that the study hypothesis may not provide guidance on where to draw boundaries but to use the quantitative information by using some type of smoothing method, such as moving averages, or by putting the exposure variable into a regression model as a continuous term. The simpler approach of calculating risks directly will require a reasonably sized population within categories of exposure if it is to provide a statistically useful result. To get incidence rates, then, we need to group the experience of individuals into relatively large categories.

RECALL BIAS

Birth cohort studies – depending on the information gathered and the length of the time interval – minimise recall bias. However, parents were asked at birth about

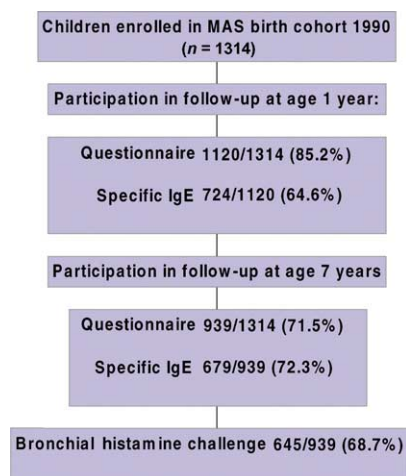


Figure 5 Participation rate at birth, rates for questionnaire data, blood samples and pulmonary function tests at age 1 and age 7 years (MAS).

symptoms of asthma, allergic rhinitis and atopic dermatitis. Two years later these questions were asked again. Fathers gave positive answers about their own atopic disease significantly more frequently at the second questioning when their offspring suffered from atopic dermatitis.¹³ This may imply that a positive family history as a risk factor for atopic disease has to be interpreted with caution in cross-sectional studies of affected and non-affected children since parents of affected children are more likely to recall symptoms of atopy in childhood than parents of non-atopic children.

QUESTIONNAIRES, INTERVIEWS, DIAGNOSTIC PROCEDURES

Validated instruments should be used to assess disease associated traits. MAS questionnaires and interviews were based on the ISAAC (International Study on Asthma and Atopy in Childhood) study questionnaires.¹⁴ This also allows the comparison of results with other internationally conducted cohort studies.

Whenever possible, questionnaire data should be confirmed by laboratory analyses. An inhalant allergen screening test (Phadiatop) was performed in a subset of parents to further confirm the diagnosis of atopy. In children, urine cotinine levels were measured, to confirm the degree of passive tobacco smoke exposure stated in interviews/questionnaires.

A high compliance was observed for procedures and tasks that did not involve physical discomfort (filling in of questionnaires, keeping diaries, collection of dust samples, urine samples, pulmonary function tests). Diagnostic procedures at follow-up visits are listed in Fig. 2.

Cord blood IgE

This was determined in a total of 6401 samples. To exclude contamination with maternal blood, IgA (which does not cross the placenta and is not detectable in cord blood) was also determined. Population-based cord blood IgE values could be described.¹⁵ However, increased levels of cord blood IgE were not shown to be a risk factor for atopic disease.¹⁶

Total and specific IgE antibodies

IgE antibodies to common food and inhalant allergens were analysed at 1, 2, 3, 5, 7 and 10 years in 60–70% of the study population. EMLA[®] cream improved acceptance and we noted that blood tests were better accepted than skin prick testing (SPT).

Total and specific IgE levels allowed the estimation of the natural course of allergic sensitisation as well as total serum IgE levels over the first 6 years of life for a population-based sample after weighting the samples for the risk factors on which the selection procedure was based (atopic family history and/or elevated cord blood IgE).^{17,18}

Urine samples

Urine samples for the determination of cotinine levels were collected at the ages of 1, 2, 3 and 4 years.

Pulmonary function tests and bronchial histamine challenges

These were performed at the age of 7 years in 645 children (71.5% of the participants at age 7; 48% of the total study population). Pulmonary function tests (PFTs) were well tolerated and compliance was satisfactory for most children. In contrast, bronchial challenges with histamine (methacholine is not approved for bronchial challenges in Germany), were strongly disapproved of mainly due to side effects (rush, nausea, vomiting) and the duration of the procedure. Furthermore, we observed that even non-atopic, healthy children showed a remarkably high degree of responsiveness to histamine with respective clinical signs.¹⁹

Dust sampling

Dust samples were collected by parents according to a detailed written instruction asking them to vacuum 1 m² in the living room, the parents' and the child's sleeping room for 3 min (1 m² in each room). This was in accordance with the recommendations of a consensus paper,²⁰ published later in 1995. In a pilot phase, we assessed the variability introduced by different field workers. The coefficient of variation ranged from 5–25% if a site was vacuumed by three different persons on different days and from 5–25% if the same person vacuumed the same site on different days ($n = 10$). The allergen concentrations in samples from the same household collected at different times correlated (Spearman's Rho $\rho = 0.7$). This variability is only slightly higher than in studies using standardised sampling schemes.²¹

Genetic studies

Longitudinally stable and extreme phenotypes (e.g. very high total serum IgE at several timepoints, early onset atopic dermatitis) can be defined and tested for linkage and associations with genetic markers or polymorphisms.^{22–25} Control groups can be selected from the equally well characterised remaining cohort. Parental DNA analyses further allow the assessment of transmission disequilibrium of alleles. When cohort studies are planned the conductance of future genetic studies should be taken into consideration when application for the Ethics committee and written informed consent forms are prepared. Since both parents are available at the inclusion timepoint in most cases, drawing of parental blood samples for later genetic analyses should be performed as early as possible. Collecting blood samples, particularly from fathers at a later timepoint has proved difficult due to a high separation/divorce rate.

INVESTIGATOR MEETINGS

All investigators (physicians, nurses, technicians, biostatisticians) should meet at regular intervals to discuss protocols for visits, to exchange experiences and publications and to discuss finances.

Present and future publications were discussed at yearly meetings in Berlin with all investigators. If new ideas occurred in between study meetings, investigators submitted a brief proposal that summarised the planned analyses and the data required.

DATA MANAGEMENT AND DATA ANALYSES

All procedures regarding data management should be standardised and fixed in guidelines. Those guidelines should be approved by the responsible data and project managers. A clear and comprehensive documentation of the study procedures is of the utmost importance, especially in the case of an external audit or change of personnel. Any changes of procedure should be justified and properly documented. Internal audits should take place to control the study process. Training of staff is another important aspect of data quality assurance. With reference to the guidelines for clinical trials (GCP) the International Epidemiological Association (http://www.dundee.ac.uk/iea/euro_Contents.htm) and the German Epidemiological Association (extended version: <http://medweb.uni-muenster.de/institute/epi/dae/>) formulated Guidelines for Good Epidemiologic Practice (GEP).

In the MAS, one data manager has been in charge of the complete dataset. Questionnaires were checked and any queries or missing data were completed by telephone where possible. If a follow-up visit was scheduled any queries or missing data were completed during the visit at the respective centre.

At the beginning of the study, data were entered on to a computer system at each of the collaborating centres. Since the results of scientific research rely on the quality of the data, the data entry process was centralised at the co-ordinating centre. Decentralised data entry can only be recommended if constant training and quality control are guaranteed. For quality assurance a random sample of e.g. 10% can be drawn and double-checked.

The database system must be a current and user friendly package so that all relevant personnel involved can handle it. For the MAS, the database system MS-ACCESS has sufficient properties for programming of data entry masks, data entry performance and plausibility checks. Data editing, data entry and validation should be done continuously by the co-ordinating centre. Automated plausibility checks such as defining ranges of plausible values for variables, listing of outliers and other logical controls have been very important for assuring high data quality.

Due to the enormous amount of information collected in the MAS and the increasing number of hypotheses

developed by all participating collaborations, MAS data have been analysed in three institutions. The methods used for the measurements of the endpoints, both subjective and objective, should be validated and meet standards for accuracy, precision, reproducibility, reliability and responsiveness (sensitivity to change over time). Problems may arise with the measurements of laboratory variables due to developments in measurement techniques. The objective(s) and hypotheses should be clearly stated before starting the study. However, the study should be able to include additional objectives for new hypotheses arising through new scientific knowledge.

Due to the non-randomised design of a (birth) cohort study statistical analyses use multivariate analysis techniques and adequate adjustment for potential confounding. Those models, such as multiple (logistic) regression models²⁶ or Cox proportional hazards models²⁷ are used to estimate the independent effect of various (risk) factors on the outcome variable. The Cox model has several advantages. It accounts for the variable duration of follow-up, the censoring of subjects and time-to-event.²⁸ One advantage of longitudinal studies with repeated measurements of outcome and explanatory variables is that it can allow improved insight into the course of disease. However, those repeated observations are correlated and longitudinal data analysis has to consider the dependency of the data. Longitudinal regression techniques can be used (e.g. generalised estimating equations (GEE) and random effects models (REM) in contrast to naive techniques that ignore the dependency of the observations.

In the MAS, one biostatistician has been in charge of the complete dataset and data transfer. Due to the enormous amount of information collected and the increasing number of hypotheses of investigators, MAS data have been analysed in three different biostatistics institutions. However, definition of disease-associated traits, variables used and statistical approaches have remained consistent.

SUMMARY AND CONCLUSIONS

Birth cohort studies require long-term interdisciplinary collaborations. Validated instruments should be used to assess disease-associated traits. Staff should be employed long-term. Hypotheses should be clear prior to commencing the study; however, the study should be open to include additional objectives. To perform adequate statistical analysis the number of participants should be as high as possible and drop out rates should be as low as possible.

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