INTRODUCTION

The “materials and methods” section of a scientific paper is the second component of the conventional IMRAD (Introduction, Materials and methods, Results and Discussion) structure of an original article. However, minor variations often exist among different journals; and this section may also be known by other names such as Subjects and Methods, Patients and Methods, Methodology, or simply, Methods. Authors should always check the individual journal’s “Instructions to Authors” or “Author Guidelines” for details of in house style requirements.

The “materials and methods” section is probably the most important part of a manuscript, as fundamental flaws in this section will invariably lead to rejection during the peer review process. The main purpose of the “materials and methods” section is to describe the study in sufficient detail such that other competent researchers are able to repeat the study, based on their reading of this section. The components of the “materials and methods” section should address the following questions:

- What was done?
- How was it done?
- How will the data be analyzed?
- Which type of study, location of study and period and duration of study?

Ideally, the “materials and methods” section should be written before the start of the study, i.e. during the planning stage. It makes sense for the author and research team to refine and perfect the way the study should be conducted before embarking on it as good research should be well justified, well planned and appropriately designed enough to address the research question. It is also recommended that the input of
a biostatistician be sought during this study design stage, so that statistical issues, including power calculations, are resolved prior to the start of the research. A prestudy “materials and methods” section may also be part of a grant proposal.

When the study has been completed, the “materials and methods” is usually the first section to be written during manuscript preparation. The way the study was actually done, starting with the research plan, how the subjects were recruited (or how the materials were obtained), and the various methods used to obtain the data should be described in chronological order. The findings for all the items included in this section should also subsequently appear in the results section. Passive voice and third person in the past tense is recommended for writing this section.

**MATERIALS**

How were the subjects recruited? Were they recruited prospectively or collected retrospectively? (Example 1). The subjects should include patients, normal volunteers or animals, as well as controls. The source population should be defined, and the sampling method used described in detail. Both the inclusion and exclusion criteria used for recruitment of the study group should be clearly stated (Example 2). For selection of the control group, how they relate to the study group should be described, e.g. matched by age, gender, ethnicity, clinical condition (Example 3). Details are important. For animal subjects, details such as genus, species and strain; age, gender, nutritional state; physiological or pathological status (e.g. pregnant, castration); rearing method; diet (e.g. constituents and sources) and name of supplier are expected (Example 4).

Medical research involving human studies should be performed according to principles outlined in the World Medical Association Declaration of Helsinki (59th WMA General Assembly, Seoul, 2008). Approval from a formally-constituted review board (Institutional review board [IRB] or ethics committee) is required for all studies involving humans, medical records, and human tissues. Informed consent from participants of the study should always be sought; if this is not possible, the IRB should decide whether this is ethically acceptable (Example 5). The IRB may also waive the requirement for informed consent, particularly for retrospective studies or case record reviews. Animal experiments require compliance with ethical and existing regulatory principles, and local licensing arrangements and guidelines. Statements indicating approval from the IRB, institutional animal care
committee or other appropriate bodies; and whether or informed consent was obtained or waived, should be provided.

**METHODS**

As reproducibility of the study methodology is vital, complete details of new or modified methods, precision of measurements and statistical analysis should be provided. For apparatus or equipment, model details, manufacturer and city of manufacture should be stated (Example 6). Any modifications made to equipment or construction of new equipment should be described, and if necessary, illustrated by photographs or diagrams. For drugs or chemicals, the exact dosages, route of administration, generic name, supplier's name, and chemical name for non-standard drugs, should be provided. The exact types, sources and supplier's name should be provided for tissues, tissue cultures, cell lines, immune sera, bacterial cultures and viruses, culture media and buffers, and reagents (Example 7).

The evaluation methods used should be comprehensively described, e.g. number of observers, whether they were blinded or not, whether assessments were done independently or by consensus, and if done, the exact time period between readings (Example 8). Was the evaluation prospectively or retrospectively performed? Was a grading system used? Were evaluations recorded on specially-designed forms? If so, all the items should be listed and if relevant, this form may need to be included as an appendix. Intra- and inter-observation variations may need to be calculated.

The method of proof should be clearly stated. This includes surgery, biopsy, histology (Example 9) and other established methods such as blood and specimen cultures, and biochemical tests (Example 10). Absence of disease on follow-up and the duration of follow-up should also be documented, if relevant.

**STATISTICAL EVALUATION**

Which test was used, why was particular test chosen, on what data, to determine what? Enough detail should be provided so that results can be independently verified. Ideally, standard statistical methods should be used. For standard tests, provide name, version, company, and city (Example 11). If not well known, the test should be described in detail. For advanced or unusual tests, a reference should be provided. It is good practice to again seek the advice of a biostatistician during manuscript preparation.
COMMON PROBLEMS

- Insufficient details of methodology, i.e. not specific and not comprehensive
- Misplaced information, e.g. results appearing in the "materials and methods" section, and vice-versa
- Wrong statistical test used
- Providing irrelevant information
- Noncompliance with journal’s "instructions to authors".

SUMMARY

The "materials and methods" section should state clearly how the study was done, how the data was collected and how it was analyzed. Above all, reproducibility is the key. To achieve this, this section should contain sufficient detailed information to enable any researcher to replicate the study.

EXAMPLES

Example 1: Retrospective study showing how subjects were recruited*

This was a retrospective study consisting of 400 patients who had undergone an appendicectomy between October 2006 and May 2008 and who were identified from the operation note database of the Department of Surgery, Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, Brunei Darussalem.

Example 2: Definitions of inclusion and exclusion criteria**

The inclusion criteria were infants with respiratory distress, an oxygen index (OI) ≥ 25 despite HFOV support (Sensormedic high frequency oscillator. 3100A. Yorba. Linda. CA. USA) and echocardiographic evidence of PPHN. The echocardiographic features of PPHN were a

---


normal cardiac anatomy with right-to-left shunt at the foramen ovale and/or ductus arteriosus, with or without dilatation of the right ventricle. The exclusion criteria were infants with lethal congenital anomalies (except congenital diaphragmatic hernia), substantial bleeding diathesis (e.g. massive intracranial hemorrhage, intraventricular hemorrhage ≥ Grade 3, platelet count < 50,000/L), active seizures, blood pressure that could not be stabilized, or gestational age < 34 weeks.

**Example 3: Selection of control and study groups**

The rats were randomized and divided into four groups. Groups 1 and 2, the control groups, comprising six young rats and six adult rats, respectively, were injected with saline. Groups 3 and 4, the injury groups, also comprising six young rats and six adult rats, respectively, were injected with FeCl₃. All the rats were observed for six hours post-injection for seizure events, after which they were killed and decapitated. Their left hemispheres were extirpated and tested for the MDA levels and SOD activities.

**Example 4: Details of animal subjects, including ethical approval**

Adult male Wistar rats whose body weight ranged from 150 to 160 g were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, India. They were housed in an environmentally controlled room that was maintained at a temperature of 22°C ± 2°C and humidity 55% ± 5, with a 12-hour light/dark cycle. The animals received a standard pellet diet (Karnataka State Agro Corporation, Bangalore, India) and tap water ad libitum. They were cared for according to the principles and guidelines of the Institutional Ethical Committee of Animal Care, Rajah Muthiah Medical College and Hospital, Annamalai University, and all treatment procedures were approved by the Committee.


Example 5: Ethical approval and informed consent from parents*

This was a randomized controlled trial carried out at the neonatal intensive care unit of the Hospital Universiti Kebangsaan Malaysia over a 37-month period, between 1 April, 2000 and 30 April, 2003. The study protocol was approved by the hospital scientific and ethics committees. Written parental informed consent was obtained before randomization.

Example 6: Details of equipment used**

All CTPA examinations were performed with multi-detector scanners using a standard protocol. The CT machines were either Somaton Sensation 16 or Somaton Sensation 64 (Siemens, Erlangen, Germany). Intravenous iodinated contrast agent (Omnipaque 350) was delivered at a rate of 3 ml/sec via mechanical injectors, either Stellant (Medrad, PA, USA) or Dual Shot (Nemoto, Japan). A total of 90 ml of contrast was administered.

Example 7: Details of fluorescence in situ hybridization (FISH) analysis***

FISH was performed on touch imprints from fresh tumor samples and fixed immediately in modified Carnoy’s fixative (3:1 methanol/glacial acetic acid). Hybridization and wash protocols were performed as described elsewhere. The slides were counterstained with 4’6-diamidino-2-phenylindole (DAPI) in antifade solution (Vectorshield, Vector Laboratories, Burlingame, CA, USA). The FISH preparations were analyzed under an Olympus BX 60 fluorescence microscope equipped with filter sets for DAPI, FITC, rhodamine, dual band-pass for FITC/rhodamine and tripe band-pass for FITC/rhodamine/DAPI. Images were acquired via a CCD camera (COHU) and digitized and processed with Powersgene MacProbe Imaging software (Applied

---


Imaging, Newcastle-upon-Tyne, UK; now Genetix Ltd). At least 100 interphase cells were scored for MYCN status and 50 to 200 cells were scored for 1p and 17q statuses. For one case (Patient 10), 1p deletion and 17q gain studies by FISH were not performed because of insufficient cells and a severely crushed tumor.

Example 8: Evaluation of ultrasonographical images and evaluation criteria*

AU subjects underwent ultrasonographical examination of the gall bladder and common bile duct during the study period. The SCI patients were examined 108 ± 25 days after the trauma and all cases within the six months from the injury onset. Ultrasonographical examinations were performed using two echo units (HDI 5000 and HDI 3500, ATL Ultrasound Inc, Bothell, WA, USA) equipped with a high-resolution 2 to 5 MHz curved transducer. The examinations were carried out by three independent experienced radiologists blinded to the patients' identities, and the images were interpreted in consensus. For each ultrasonographical session, the transducer was placed over the right upper abdominal quadrant. Biliary sludge was defined as non-shadowing low-amplitude echoes layering in the dependent portion of the gallbladder or common bile duct and forming a fluid-fluid level with changes in the patient position. Gallstones were defined as echogenic intraluminal filling defects of the gallbladder or common bile duct with an accompanying posterior acoustic shadow, moving freely with gravity. In cases of lithiasis, the size of the gallstones was measured. The gallbladder wall thickness and echogenicity, as well as the bile duct width were registered in all subjects.

Example 9: Proof of diagnosis by histological criteria**

For the diagnosis of FA, colloid-filled follicles having uniform-appearing epithelial cells together with a well-confined capsule formation were identified. Careful observations to exclude malignancy and to differentiate from NG were performed. In HA, the lesions composed of


cells with abundant eosinophilic cytoplasm and small regular nuclei were taken into account. For NG, thyroid nodules containing colloid-rich follicles lined by flattened, inactive epithelium were noted. TG was diagnosed by the presence of crowded glands and follicles lined by tall columnar epithelia. The enlarged epithelial cells project into the lumens of the follicles and the scalloped appearance of the edges of the colloid are diagnostic. In HT, the thyroid parenchymas with a dense active lymphocytic infiltration are diagnostic.

Example 10: Proof of diagnosis using biochemical criteria*

The diagnosis of adrenal insufficiency was made using the following criteria: baseline cortisol levels of < 550 nmol/L; cortisol response following LDT, increment of cortisol < 250 nmol/L and peak cortisol <700 nmol/L; and following SDT, increment of cortisol < 250 nmol/L \(^{(18)}\) and peak cortisol < 938 nmol/L. Statistical analysis was performed using the Wilcoxon rank test for repeated measurements. The Mann-Whitney test was used to determine the significance between two groups (survival and nonsurvival) and for numerical variables. A p-value of < 0.05 was deemed to be of statistical significance.

Example 11: Description of statistical tests**

Statistical analysis was performed using the Statistical Package for the Social Sciences 13.0 version for Windows program (SPSS Inc, Chicago, IL, USA). The continuous variables are described as average ± standard deviation and median, interquartile range. The categorical variables were presented in terms of their frequency. For a comparison of the means with a normal distribution between the patient groups, student's t-test and one-way ANOVA were used as parametric tests. For a comparison of the means without a normal distribution between the patient groups, Mann-Whitney U-test and Kruskal-Wallis test were used as nonparametric tests. The presence of differences was tested using the Mann-Whitney U-test, and the source of the difference was found


with the Kruskal-Wallis test. In order to compare the categorical variables between the patient groups, Pearson's chi-square, Fisher's exact, Kolmogorov-Smirnov and Mantel-Haenszel chi-square tests were used. After the normality assumptions were assessed, a two-way mixed design ANOVA (with independent measures on mortality) was performed with the Greenhouse-Geisser adjustment. The relationships between patient characteristics and survival were analyzed by the Kaplan-Meier and Cox Regression Analyses (Forward LR). A p-value of less than 0.05 was regarded as significant.

**SUGGESTED READING**