

WRITING THE MATERIALS AND METHODS

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LECTURE OUTLINE

- Introduction
- Materials
- Methods
- Common problems
- Summary



INTRODUCTION

Materials and methods section

- Also known as
 - Subjects and methods
 - Patients and methods
 - Methodology
 - Methods



BODY OF PAPER

IMRAD

- Introduction (What question was asked?)
- Methods (How was it studied?)
- Results (What was found?) And
- Discussion (What do the findings mean?)



INTRODUCTION

Materials and methods section

- Most important part of paper
- Flaws in this section
 - will lead to rejection



INTRODUCTION

Purpose of M&M

- Describe study in sufficient detail
- Other competent researchers are able to repeat the study
- Details are important
- Like a cooking recipe!



INTRODUCTION

Components of M&M

- What was done?
- How was it done?
- How was the data analysed?
- Do not include data obtained during course of study (Results)



INTRODUCTION

Write M&M section early

- Ideally before start of study
- Consult statistician during study design stage
- May be part of grant proposal



INTRODUCTION

Materials and Methods

- Usually the first section to be written during manuscript preparation
- Description of how study was conducted - Use past tense



MATERIALS AND METHODS

Sequence

- Chronological order in which study was actually done
 - research plan
 - materials and subjects
 - methods used



MATERIALS

- How subjects were recruited
 - patients, animals, controls
- Define
 - source population
 - inclusion criteria
 - exclusion criteria



METHODS

This was a retrospective study consisting of 400 patients who had undergone an appendectomy 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 Darussalam. The inclusion criteria

Retrospective subject recruitment

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 haemorrhage, intraventricular haemorrhage \geq Grade 3,¹⁰ platelet count $<$ 50,000/L), active seizures, blood pressure that could not be stabilised, or gestational age $<$ 34 weeks.

Examples from SMJ

Inclusion and exclusion criteria



MATERIALS

- Details are important
- Animals
 - genus, species, strain
 - age, gender, nutrition, physiological and pathological status




The rats were randomised 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.

Adult male Wistar rats whose body weight ranged from 150-160 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.

Selection of control and study groups

Examples from SMJ

Details of animal subjects



MATERIALS

- Ethical approval is mandatory
 - WMA Declaration of Helsinki 2000
 - institutional/national committee approval
 - informed consent
 - guidelines for animal experiments




Materials and Methods

Animals, Anesthetics, and Preparation

The protocol of this study was approved by our institutional animal care and use committee. Eleven femurs of seven female dogs (weight range, 20-25 kg) were treated under general anesthesia. All seven dogs were anesthetized using an intramuscular injection of 50 mg/kg ketamine hydrochloride and 5 mg/kg of xylazine (Rompun, Bayer Korea). Booster injections of up to one half the initial dose were administered as


Subjects and methods

This study included 27 infants with extrahepatic biliary atresia who were studied prospectively starting at the time of initial diagnosis. They were attendants of the Hepatology Clinic of the New Children's Hospital, Cairo University. Parents of the participating infants gave their consent to the trial. The study commenced by May 2001 and ended by January 2004.



METHODS

- Reproducibility is vital
 - complete details of new methods
 - precision of measurements
 - appropriate statistical analysis



Introduction

Febile neutropenia is a serious complication to patients with solid tumors and hematological malignancies. Such complication can be severe and occasionally fatal. Although the mortality associated with febile neutropenia has dramatically decreased over the past 3 decades, the overall death rate during or immediately after an episode of febile neutropenia can be as high as 10% with half of the patients dying directly as a result of infection itself¹. Much has changed in the patterns of microbial flora and the drugs used. Gram-positive organisms are becoming more common than gram-negative ones as causes of bacteremia²⁻⁴. This study was designed to review the patterns of febile neutropenia presentations, patterns of microbial flora and other characteristic features in cancer patients with solid tumors. This will assess many factors in our institute which may lead to modifications in our practice and guideline recommendations for better outcomes and improvement in morbidity and mortality.


Methods

Between November 1999 and December 2003, data of all patients admitted to King Abdul Aziz University Hospital (KAUH) with febile neutropenia in ST were collected and analyzed. All characteristic features were analyzed by using simple descriptive statistical analysis (frequency distribution, cross tabulation, chi-square and Fisher's exact test) by SPSS statistical program and were correlated with treatment outcome.

Results

Sixty seven febile neutropenia episodes in 56 cancer patients with solid tumors were studied. All characteristic features were summarized in (Table-I). Almost two thirds (61.2%) were <30 years of age while 38.8% >30 years. Males were 38.2% while females 61.8%. Sexes were 35.5% while non sexes were 64.5%. Duration of neutropenia was <7days in 92.5%, 7-14 days in 7.5% and none more than 14 days. Only 16.4% presented with severe neutropenia (ANC < 100/MCL). Focuse cultures were found only in 11 patients (16.4%). Organisms isolated, 5 (7.5%) gram-positive bacteria, 5 (7.5%) gram-negative bacteria and fungal infection in 1 (1.5%). Medical co-morbidity was found in 25.4%. Patients were stratified in to, high (7.5%) when ANC < 100/MCL, prolonged neutropenia > 14 days, significant co-morbidity or poor performance status, intermediate (44.7%) when

Bad example





Longer subdivided M&M section for complex studies

METHODS

- Apparatus/equipment
 - model, manufacturer, protocol
- Drugs/chemicals
 - exact dosages, mode of administration, generic name, formula
- Treatment details

Radiofrequency Ablation

Thermal ablated regions were created using a 500 kHz, 200-W radiofrequency generator (Genes CC-3, Valleylab) with a 17-gauge cooled-tip electrode. Radiofrequency power was then manually increased to 100 W and held for a total of 10 min, if the impedance increased by more than 10% of initial tissue impedance, the current output was automatically reduced to a level determined by the previously designed pulsing algorithm [22]. During the procedure, a thermocouple embedded within the electrode tip continuously measured local tissue temperature. Tissue impedance was monitored using circuitry incorporated into the generator. A peristaltic pump was used to infuse normal saline solution at 0°C into the lumen of the electrodes at a rate sufficient to maintain a tip temperature of 20-25°C. The incision was closed using absorbable sutures after electrode withdrawal.

All CTPA examinations were performed with multi-detector scanners using a standard protocol. The CT machines were either Somatom Sensation 16 or Somatom 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. The bolus tracking

Chin Orthop Relat Res (2008) 406:1010–1017
DOI 10.1007/s11999-008-0542-7

ORIGINAL ARTICLE

Knee Reconstruction with Preservation of the Meniscus in Tibial Giant Cell Tumor

We performed anterolateral or anteromedial incision of the knee according to the tumor location. We first excised the proximal tibia (in resection of the lateral plateau or middle tibia) in resection of the medial plateau) for the later strut grafting. Only one incision was needed with lateral condylar resection, but an additional incision was needed for medial condylar resection. We incised the joint capsule semicircularly along the attachment at the edge of the proximal tibia. The joint capsule, ipsilateral meniscus, and collateral ligament were retracted upward, ensuring preservation of the blood supply of the articular capsule and synovial lining and an intact meniscus. We specifically avoided damage to the meniscus to minimize the risk of subsequent degeneration or necrosis. We circumferentially excised the tumor in normal tissue extending to the tibial tubercle anteriorly and the midline of the tibial plateau posteriorly (Fig. 4).

The amount of tibial plateau and lesion removed was based on the extent seen on CT and MRI while preserving the tibial tubercle (the attachment point of patellar tendon) and the attachments of the anterior and posterior cruciate ligaments at the spinous process of the tibia. If the tumor had reached the midline of the tibia transversely, we performed an osteotomy deep into normal bone tissue under the tibial tubercle and spinous process of the tibia, achieving complete resection while preserving the ligamentous structures.

We obtained an iliac bone crest graft through an osteotomy in the anterior iliac wing, using separate instruments, and trimmed it to match the remaining plateau. With the iliac bone plate's concavity upward and in contact with the remaining tibial plateau, the iliac graft was placed to best conform to the radius of the femoral condyle (Fig. 5). We fixed the iliac plate to the contralateral plateau with a bolt, long cancellous screw, or steel plate, ensuring it was at the same elevation as the remaining plateau. We divided the segmental fibulae into several portions and inserted them longitudinally under the reconstructed tibial

Fig. 4 An intraoperative photograph shows the preserved meniscus and resected lateral plateau.

METHODS

Evaluation

- Comprehensive description
 - number of observers
 - blinded or unblinded
 - by consensus or independent
 - time period between readings

METHODS

Evaluation


- Prospective or retrospective
- Grading system
- Any specially-designed forms?
 - items assessed: intra- and interobserver variation
- appendix ?

Reading and Evaluation Protocol

All reads were evaluated by three experienced observers in independent sessions. A subset of 10 reads was used to train the observers to use the rating scales with focus on MR images and wrist anatomy. The independent interpretations were performed 2 months later.

For all evaluations, soft copies were interpreted with bright monitors (20" candela, Siemens Medical Solutions) on a standard PACS reporting station (MagicView 1000, Siemens Medical Solutions). Fifteen criteria were used to evaluate gross anatomy and fine anatomic detail, and a general score was given for overall anatomic impression, and for technical artifacts [14, 18-20]. For better evaluation of coronal reconstructions, epiphyseal and cortical bone was examined in-plane along the x- and y-axes and along the table feed (z-axis).

For gross carpal anatomy, we evaluated trabecular structure in-plane (x- and y-axes, axial images), trabecular structure along table feed (z-axis, coronal images), trabecular outline in-plane (x- and y-axes, axial images), trabecular outline along table feed (z-axis, coronal images), cortical thickness in-plane (x- and y-axes, axial images), cortical thickness along table feed (z-axis, coronal images), smoothness of cortical outline in-plane (x- and y-axes, axial images), and smoothness of cortical outline along table feed (z-axis, coronal images). For fine carpal anatomy, we evaluated cartilage, junctional zone of cartilage and bone, insertions of radiocarpophanate ligament, intrinsic capitate-hamate ligament, nutritive canals of hamate base, nutritive canals of capitate base, and conspicuity of ganglia and bone cysts. Other general evaluation criteria included technical artifacts not related to parameter settings and overall anatomic impression.



Chin Orthop Relat Res (2009) 466:3034-3043
DOI 10.1007/s11999-009-0511-1

ORIGINAL ARTICLE

Instrumented Posterior Lumbar Interbody Fusion in Adult Spondylolisthesis

was 0.623 and the proportion of agreement was 92%. In some patients, dynamic flexion-extension lateral views were taken when the two surgeons could not determine or reach consensus regarding the fusion status on the standing view radiograph.

We evaluated clinical outcomes preoperatively and postoperatively with the Oswestry disability low back pain questionnaire (ODI, 0%-100%), considered an accepted validated tool of low back pain functional outcome [15], and a visual analog scale for back pain (VAS; 0-10 points). The evaluation was performed at 3-, 6-, and 12-month intervals after surgery and then at 1-year intervals thereafter. We determined absence of radiolucency of the lower leg by the absence of pain or tenderness after surgery and at the one-patient visit. Patient satisfaction was rated with a four-point Likert scale (1=1) (1 = very satisfactory; 2 = satisfactory; 3 = unsatisfactory; 4 = very unsatisfactory). This was obtained from the patient's statement in the presence of an evaluator who was not involved with the surgery.

We compared preoperative and postoperative ODI and VAS scores with a paired t test and multivariate robust regression analysis (MVRRA) with preoperative function score and dummy variables for groups as covariates. Preoperative and postoperative disc height ratios, slip ratios, and segmental lordosis were assessed using a paired t test. Finally, patient satisfaction and radiolucency improvement rates were evaluated using the chi-square test. Clinical and radiographic outcomes also were assessed by MVRRA, with age, gender, fusion status, spondylolisthesis level, spondylolisthesis type, final correction of disc height ratio, final correction of vertebral slip ratio, final correction of regional lordosis angle, and dummy variables for groups as covariates. We used the Stat® program (StatCorp LP, College Station, TX) for analysis.

Results


Although the BC and AC groups (including the TC and PC groups) showed functional improvement in ODI and VAS scores after PLIF (Table 2), the TC and PC groups had greater ODI improvement than the BC group ($p = 0.008$).



METHODS

Evaluation

- Proof
 - surgery
 - biopsy
 - histology
 - follow-up and duration




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 parenchyma with a dense active lymphocytic infiltration are diagnostic.^[20]

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^[27] and following SDT, increment of cortisol < 250 nmol/L^[28] and peak cortisol < 938 nmol/L^[29]. 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 non-survival) and for numerical variables. A p-value of < 0.05 was deemed to be of statistical significance.

Proof of diagnosis using biochemical criteria

Proof of diagnosis by histological criteria


Examples from SMJ



METHODS

Statistical evaluation


- Which test used?
- Why it was chosen
- On what data?
- To determine what?



METHODS

Statistical evaluation

- Enough detail so results can be independently verified
- Ideally: use standard statistical tests
- Provide details of tests
 - name, version, company, location



METHODS

Statistical evaluation

- If not well-known statistical test, describe test in detail
- If advanced or unusual test, provide a reference



METHODS

Statistical evaluation

- Seek advice of biostatistician
 - during study planning stage
 - many variables
 - number of observations, etc
 - also during manuscript preparation



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 \pm 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 analysed by the Kaplan-Meier and Cox Regression Analyses (Forward LR). A *p*-value of less than 0.05 was regarded as significant.

Example from SMJ



MATERIALS AND METHODS

Common problems

- Inappropriate subject population
- Bias not controlled for
- Insufficient number of subjects
- No inclusion/exclusion criteria



MATERIALS AND METHODS

Common problems

- Insufficient details of methodology i.e. not specific
- Wrong statistical test used
- Misplaced information
- Instructions to authors
 - noncompliance



SUMMARY

Materials and Methods

- State what was done
- How it was done
- How the data was collected
- How the data was analysed

Above all: reproducibility

