논문작성 워크숍:

재료 및 방법, 결과 다듬기

노 주 원

동국의대

재료 및 방법
(Material & Methods)

재료(대상) 및 방법

- 목적
  - 결론에 이르기 위하여 실험을 어떻게 하였는지 보여준다.
  - 독자가 연구의 타당성을 판단할 수 있게 한다.
- 독자는 ***안*** 읽어도, reviewer는 세심하게 읽는다!
  - 실험이 잘못되었거나, 불충분하거나, 비전문적이라고 생각하면 reject 할 가능성이 높다.

재료(대상) 및 방법 작성의 개요

- "아무리 자세해도 지나치지 않다"
  - 다른 연구자가 이 연구를 평가하고 재현할 수 있도록 자세하게
- 포함될 내용
  - 연구디자인
  - 연구상태나 조건의 정의 (질병, 생리학적 상태..)
  - 연구대상의 정의 (환자, 정상인, 동물, 식물, 세포주..)
  - 연구대상 선정방법 계획
  - 구체적인 실험방법 결정
  - 모든 관찰항목과 관찰방법의 구체적인 결정
  - 자료평가를 위한 통계학적 분석법 선택과 기술

구성 (Organization)

- 주제별로 구분하고 소제목을 붙임.

Animal Studies	Clinical Studies
Materials	Study subjects
Animals	Inclusion criteria
Preparation & model establishment	Exclusion criteria
Study design	Study design
Interventions	Interventions
Methods of measurement	Methods of measurement
Calculations	Calculations
Analysis of data	Analysis of data

Clinical Study

연구의 궁극적 대상

- 목표로 하는 질환이나 상태
  - 난소암
    - 난소암 중 mucinous type 만…
    - 난소암 중 advanced stage 만?
  - 자궁경부암?
    - 수술대상의 초기…
    - Recurrent ?
- Example

1. Study subjects

This study was conducted prospectively in patients with cervical cancer the International Federation of Gynecology and Obstetrics (FIGO) stage IB1–IIA.

Clinical Study

재료(대상)의 채택기준 및 제외기준

- Inclusion criteria

  the cervical smear collected before radiotherapy in 169 patients with stage IB1 through stage IVB cervical cancer (International Federation of Gynecology and Obstetrics [FIGO]) between July 2003 and December 2006, at the National Cancer Center, Goyang, Gyeonggi, Korea.

- Exclusion criteria

  Exclusion criteria included neuroendocrine histology, pathologically proven distant metastasis, history of psychiatric disease, preoperative urinary dysfunction, and another coexisting malignancy.

Clinical Study

재료(대상) 선정방법, 규모 및 과정

- 연구에 사용한 개체 수(n)는 정확히 기록
- 시제는 과거를 주로 사용
  - "연구결과가 논문 중에 어떻게 기술되어 있다."라고 할 때는 현재 시제

  … Data are summarized as mean ± SD in Table 1…..
- 이용된 대조군 기술
- 환자를 표현할 때는 patient A, B… 등으로 표현
- CONSORT statement : for RCT
  - Consolidated Standards of Reporting Trials
  - Checklist of essential item and flow diagram
- PRISMA statement : for systematic review and meta-analysis
  - Checklist and flow chart

동물, 약제, 시료, 기구 등의 기술

- Generic name 사용
  - Paclitaxel, dopamine HCl
  - 시약은 화학명
  - 괄호
    - 상품명, 제조회사명, 제조일시, 제조번호
    - 기계, Kit : 회사이름, 소재도시명, 나라이름
    - 체중, 농도, 용량 등은 괄호로 넣거나, 앞으로 가면 괄호 없이 기술

DMEM culture medium (Gibco BRL, Long Islands, NY)

10 mg nitoglycerine , nitroglycerine (10 mg)

- 동물을 사용할 경우, 어떤 실험동물과 연령을 정확히 기술
  - Animal (X)
  - Six weeks old female athymic nude mouse….
- 측정단위 : SI Unit

방법(Methods)

- **어떻게 했는가?**
- **왜 했는가?**

Methods: 무엇을 했는가?

- 질문 (목적)
- 독립변수와 측정값 (종속변수)
- 대조군(controls)
- 각 실험의 구성, 순서(개입, 측정, 실험), 기간, 샘플 규모, ***반복실험 (repeats for reproducibility)***

Animal Study

예문: 연구디자인 (1)

- A ; 대상동물 (animals)
- B : 관리방법 (preparation)
- C : 동물모델 생성방법 (methods for model establishment)

Establishment of orthotopic tumor model

[A] Female athymic nude mice (NCr-nu) were purchased from the National Cancer Institute-Frederick Cancer Research and Development Center, and housed in specific pathogen-free conditions. [B] They were cared for in accordance with guidelines set forth by the Association for Assessment and Accreditation of Laboratory Animal Care International and the U.S. PHS Policy on Humane Care and Use of Laboratory Animals, and all studies were approved and supervised by the MD Anderson Cancer Center Institutional Animal Care and Use Committee.

[C] To produce tumors, Hec-1A and Ishikawa cells (both 4.0 x 10^6 cells per 50 μL HBSS) or Spec-2 cells (2.0 x 10^6 cells per 50 μL HBSS) (25) were injected into the mice. Before injection, mice were anesthetized with isoflurane inhalation (Baxter, Deerfield, IL), and a 0.5-cm incision was made in the right lower flank to optimize exposure to the right uterine horn. The distal portion of the horn was then identified and pulled to the incision for exposure. A single-cell suspension of 50 μL was then injected into the lumen of the uterine horn. The injection site was closely monitored during and following injection to ensure that no spillage occurred into the peritoneal cavity.

Animal Study

예문: 연구디자인 (2)

- A : 질문, 기다린 기간
- B ; n
- C; 시험군, 대조군
- D; 처치 (Intervention)
- E : 실험기간
- F : 종속변수 (dependent variables)

Therapy for established uterine tumors in nude mice

[A] To assess tumor growth, treatment began two weeks after injection of tumor cells. Mice were randomly divided into [B] 4 groups (n = 10 mice per group); [C] (a) control PBS, (b) 3G3, (c) paclitaxel (Ishikawa, Hec-1A and OVCA432) or docetaxel (Spec-2), and (d) 3G3 combined with chemotherapy (paclitaxel or docetaxel).

[D] Antibody 3G3 was dosed using 60 mg/kg intraperitoneal injection twice weekly with an initial loading dose of 214 mg/kg (21). Chemotherapy was injected into the peritoneal cavity once a week at a dose of 100 μg/mouse (paclitaxel) or 30 μg/mouse (docetaxel). Mice were euthanized after they became moribund (typically [E] six to seven weeks, depending on tumor cell type). [F] Tumor weight, number of tumor nodules, and distribution of tumors were recorded. Tumor tissue used in this study was obtained at the time of necropsy, and immersed in optimum cutting temperature medium for frozen slide preparations. Tumor specimens were also fixed in formalin for paraffin slide preparation.

실험실 연구에서 반복실험의 기술


Cancer Therapy: Preclinical

Clinical Cancer Research

Biologic Effects of Platelet-Derived Growth Factor Receptor α Blockade in Uterine Cancer

Ju-Won Roh[1,5], Jie Huang[1], Wei Hu[1], XiaoYun Yang[1], Nicholas B. Jennings[1], Vasudha Sehgal[6], Bo Hwa Sohn[6],

Figure 3

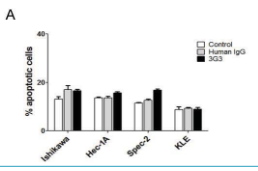


Fig. 3. Effect of 3G3 on tumor cell apoptosis. A, the apoptotic rate of cultured cell lines with treatment of 3G3 alone at 20 μg/mL, and B, the apoptotic rate after 3G3 treatment combined with cytotoxic chemotherapy in Ishikawa, Hec-1A, Spec-2, and KLE cells. Apoptosis was measured by determining the percentage of PE Annexin V/7-AAD-positive cells at 72 hours after treatment. Results were confirmed with triplicate experiments. Error bars, SEM. *, $P < 0.05$.

- Reviewer #2 (Reviewer Comments to the Author):

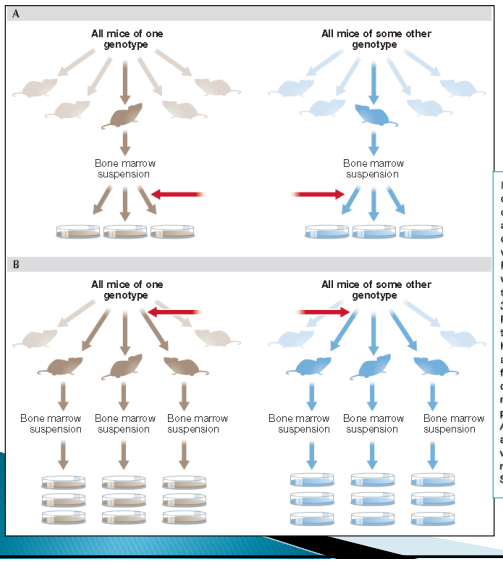
  …

  3. It is unclear from the figure legends whether experiments shown in Figs. 2, 3, and 4 reflect independent experiments or triplicate aliquots from the same experiments. This is an important issue (D.L. Vaux, EMBO Rep. 13:291, 2012). If independent experiments were performed, this must be explicitly stated. If a "representative" experiment is provided, this should also be stated.

EMBO reports

science & society

Replicates and repeats—what is the difference and is it significant?



uptake assay as described previously (24). Cells were plated on 96-well plates (7,000 per well for Ishikawa, Hec-1A, and KLE; 10,000 per well for Spec-2) in triplicate and incubated overnight at 37°C and 5% CO_2. After incubation, cells were

본문 : "Cells were plated on 96-well plates in triplicate~ "

Figure 3. Effect of 3G3 or the combination of 3G3 and chemotherapy on apoptosis. A, apoptosis of Hec-1A and RL95-2 cells was measured after treatment with 3G3 and PDGF-AA. "3G3 + PDGF-AA" means pretreatment with 3G3 before PDGF-AA stimulation, and "PDGF-AA + 3G3" means cotreatment with PDGF-AA and 3G3 at the same time. B, apoptosis of Ishikawa, Hec-1A, Spec-2, and KLE cells after pretreatment with 3G3 followed by cytotoxic chemotherapy. Apoptosis was measured by determining the percentage of PE Annexin V/7-AAD–positive cells at 72 hours after treatment. Statistical analysis was performed on the basis of 3 repeated experiments. Error bars, SEM. *, $P < 0.05$.

Legend : "~Statistical analysis was performed on the basis of 3 repeated experiments. ~"

실험방법의 기술

- 잘 알려진 방법
  - 설명 없이 참고문헌 제시
- 잘 알려지지 않은 방법
  - 핵심적인 특징 기술, 참고문헌 제시
- 개량한 방법
  - 개량한 것의 근본적인 특성과 목적 기술
- 새로운 방법
  - 완벽하게 설명필요 -> 독자들이 평가하고 재현 가능하도록

데이터분석

- 어떻게 변수를 계산하였는지
- 데이터를 어떻게 요약하였는지
  - 정규분포: 평균값과 표준편차
  - 비정규분포
    - 중앙값(median)과 범위(range)
    - 중앙값(median)과 사분위수범위(range between the 25th and the 75th percentiles)

통계 분석

- 잘 알려진 방법: 통계 방법만 기술.
  - Student t-test, Chi-square, ANOVA, linear regression, correlation, Wilcoxon
- 잘 알려지지 않은 통계 방법:
  - 논문이나 책을 참고문헌으로 제시.
- 사용한 프로그램 (version, release number 포함)
- 각 통계 방법마다 샘플 크기가 다른 경우, 분명하게.
- 유의한 p 값 또는 95% 신뢰구간

예문: 데이터분석

5. Statistical analysis

Continuous variables were assessed for normal distribution (Kolmogorov-Smirnov test) and expressed as appropriate (mean with SD or median with range). Categorical variables were evaluated with the use of t Fisher exact test. For paired data, such as postoperative changes in UDS or IPSS score compared with preoperative baseline, Wilcoxon singed rank test was used for analysis p<0.05 was nsidered statistically significant. DFS and OS were evaluated by Kaplan-Meier analysis. IBM SPSS ver. 20.0 (IBM Co., Armonk, NY, USA) was used for all statistical analyses.

1. How the data were summarized
2. Statistical test used (well known; no reference needed)
3. P value at which differences
4. Statistical program used

예문: 데이터분석

Statistical analyses

Continuous variables were assessed for normal distribution (Kolmogorov–Smirnov test) and expressed as appropriat mean with SEM or median with range). one-way ANOVA test with *post hoc* (Bonferroni adjustment) comparison or Kruskal–Wallis test with multiple comparisons (Wilcoxon rank-sum test with Bonferroni correction) was performed to determine the statistical significance as appropriate. Categorical variables were evaluated with use of the Fisher exact tes Three replicates were taken to monitor the performance of each experiment. We repeated experiments independently at least 3 times for statistical analysis. $P <$ 0.05 was considered statistically significant. IBM SPSS Statistics 21.0 (IBM SPSS, Inc.) was used for all statistical analyses.

Clin Cancer Res; 20(10) May 15, 2014

1. How the data were summarized
2. Statistical test used
3. Measurements that were compared
4. P value at which differences
5. Statistical program used

정확한 어휘 선택

- Measure, calculate, estimate의 용어 구분
  - "We measured heart rate and ventricular pressure and calculated maximal positive dP/dt."
- Determine; measurement and calculation
  - "We determined heart rate, ventricular pressure, and maximal positive dP/dt."
- Study, experiment, series, group의 용어 구분
  - Study: 현상이나 발달, 질문에 대한 지속적이고 체계적인 조사
  - Experiment: 가설의 타당성을 조사하기 위한 시험 (대상이 인간일 경우 study라고 함)
  - Series: 서로 연관된 2개 이상의 실험
  - Group: 같은 특성을 갖는 실험동물 또는 인간

관점(Point of view)

- 수동태가 많이 쓰임
  - Materials & methods 강조하기 위해
  - 글의 활력을 주기 위해 능동태를 한 번 정도 사용하기도 한다.

We collected the different fungal species from various tepuis in Venezuela.
Different fungal species were collected from various tepuis in Venezuela.

- 이유 없이 관점을 바꾸지 마라.
  - The assays were performed for 10 min at room temperature. We then added 10 ml of 95% ethanol. The assays were performed for 10 min at room temperature. The 10 ml of 95% ethanol were added.

관점(Point of view)

- We로 시작하는 문장이 너무 많아지지 않게
  - 하나의 실험의 단계를 한 문장에 넣음.
    - We dehydrated the pellets, cleared them with propylene oxide, and embedded small pieces of each pellet in blocks of Spurr's resin.
- 앞 부분에 변화를 주는 방법.
  - After 30 s, we centrifuged the samples.
  - Then we centrifuged the suspension as before.
  - To prepare isolated surface layers for electron microscopy, we resuspended the 0.1-ml pellets of packed, …

재료 및 방법에서 흔히 보이는 오류

- 필요한 내용이 빠지는 경우 (방법과 결과가 일치하지 않는 경우)
- 특정 실험을 왜 했는지 알 수 없는 경우
- 특별한 이유 없이 수동태에서 능동태로
- 특별한 이유 없이 과거시제에서 현재시제로

재료 및 방법 기술의 지침

1. 훈련된 연구자라면 연구를 재현하기에 충분한 내용과 참고문헌을 기술하되, 불필요한 세부사항을 포함하지 않는다.
2. 재료 및 방법 이외에 결과를 포함하지 않는다.
3. 긴 설명이 필요한 세부사항은 부록을 활용한다.
4. 적절한 주제 또는 소주제 별로 내용을 정렬한다.
5. 새로운 주제는 적절한 신호를 사용하여 연결한다.
6. 기능이 명확하지 않은 실험절차는 그 목적을 설명한다.
7. 수동태가 바람직하다.
8. 뚜렷한 이유 없이 관점을 바꾸지 않는다.
9. 정확한 단어를 사용한다.
10. 윤리 지침을 따르고, 이에 대해 기술한다. (animal & clinical)

결과
(Results)

결과 작성의 전략 (1)

- 표와 그림을 잘 구성하고, 활용
- 결과부분은 소제목 (subheadings) 을 활용
- 각 부제목에서 각 표와 그림의 부분을 설명하고, 해당하는 표와 그림을 표기

결과 작성의 전략 (2)

- 각 소제목에서 각 표와 그림을 언급하여 설명하되, 표와 그림의 내용을 반복하는 것은 최소화
- 각 결과가 재료와 방법에서 언급된 연구방법에 의한 결과임을 확인

결과 작성 요령

1. Use past tense
2. Do not repeat methods
3. Do not interpret in depth
4. Use of Figures and Tables
5. If data are presented in tables and figures, summarize in the text
6. Highlight important findings (with summary / introductory sentence, header)
7. Use of "Data Not Shown"

Blueprint for Results

STROBE – Obs study	CONSORT – Randomized trials
Participants	Participant flow: diagram is strongly recommended
Descriptive data	Recruitment
Outcome data	Baseline data
Main results	Numbers analyzed
Other analyses	Outcomes and estimation
	Ancillary analyses
	Harms

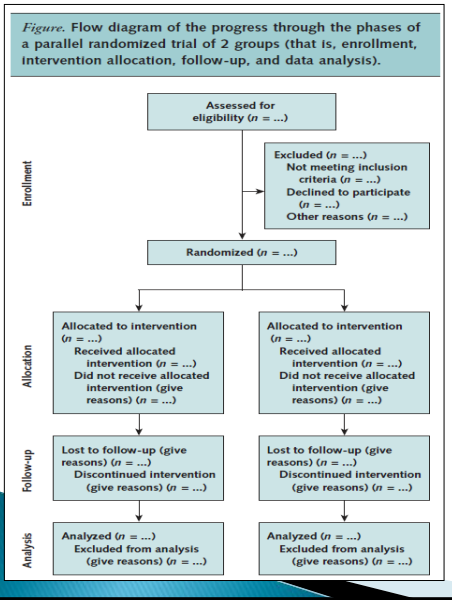
The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement

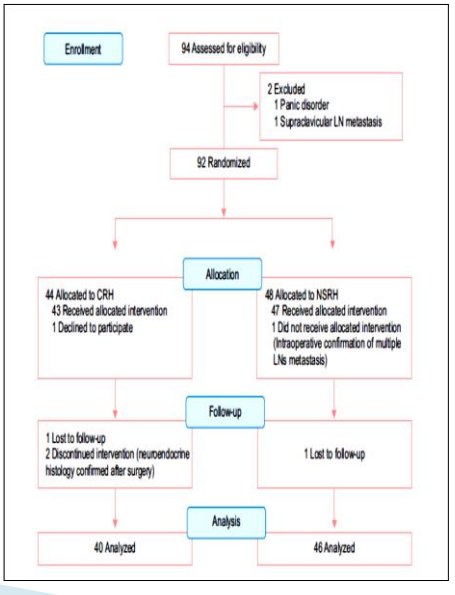
CONSORT (Consolidated Standards of Reporting Trials)

Participants (STROBE)

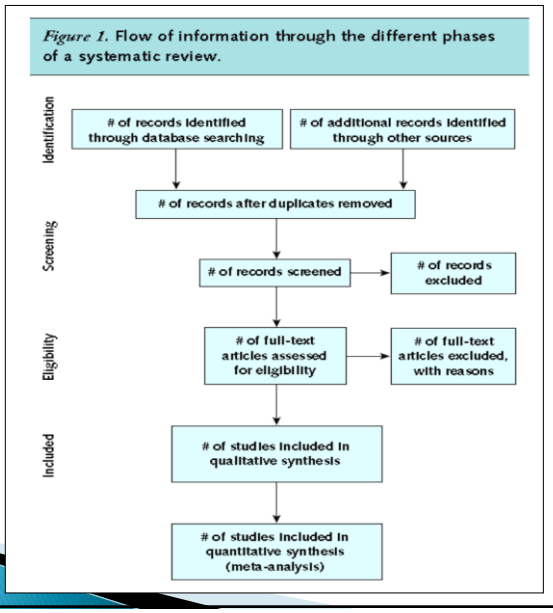
- Report the numbers of individuals at each stage of the study (ex. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed)
- Give reasons for non-participation at each stage
- Consider use of a flow diagram

Participant Flow (CONSORT)





PRISMA (meta-analysis)



Descriptive Data (STROBE)

- Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders
- Indicate the number of participants with missing data for each variable of interest
- Cohort study – summarize follow-up time (eg, average and total amount)

Outcome Data (STROBE)

- Cohort study
  - Report numbers of outcome events or summary measures over time
- Case-control study
  - Report numbers in each exposure category, or summary measures of exposure
- Cross-sectional study
  - Report numbers of outcome events or summary measures

P value의 기술

- Only written to three decimal place (eg. $P = .032$)
- When the P value is less than .001 → $P < .001$
- When the *P* value is greater than .999 → $P > .999$
- *P* value is indicated as the actual value (not displayed as "not significant" or "NS")

Summary

- 논문의 전체 구성을 늘 생각하고 작성해야 한다.
  - 시작하여 단기간에 완성하는 것이 바람직하다.
- Introduction에서 질문하고, 방법과 결과를 통해 근거를 제시하고, discussion에서 답을 하는 큰 흐름을 유지한다.
- 세세한 문법보다는 논리적인 문맥의 흐름에 집중한다. 단, confidence 강도를 결정하는 단어의 선택은 신중하게 (교정으로 바꿀 수 없는 부분들…)
- 게재를 원하는 저널을 미리 선정하고, 유사한 형식의 논문을 많이 읽어, 형식과 경향성을 파악하여 참조한다.