THE STATE UNIVERSITY OF MEDICINE AND PHARMACY "NICOLAE TESTEMIŢANU"

Department of Management and psychology

EXPERIMENTAL AND DIAGNOSTIC STUDIES

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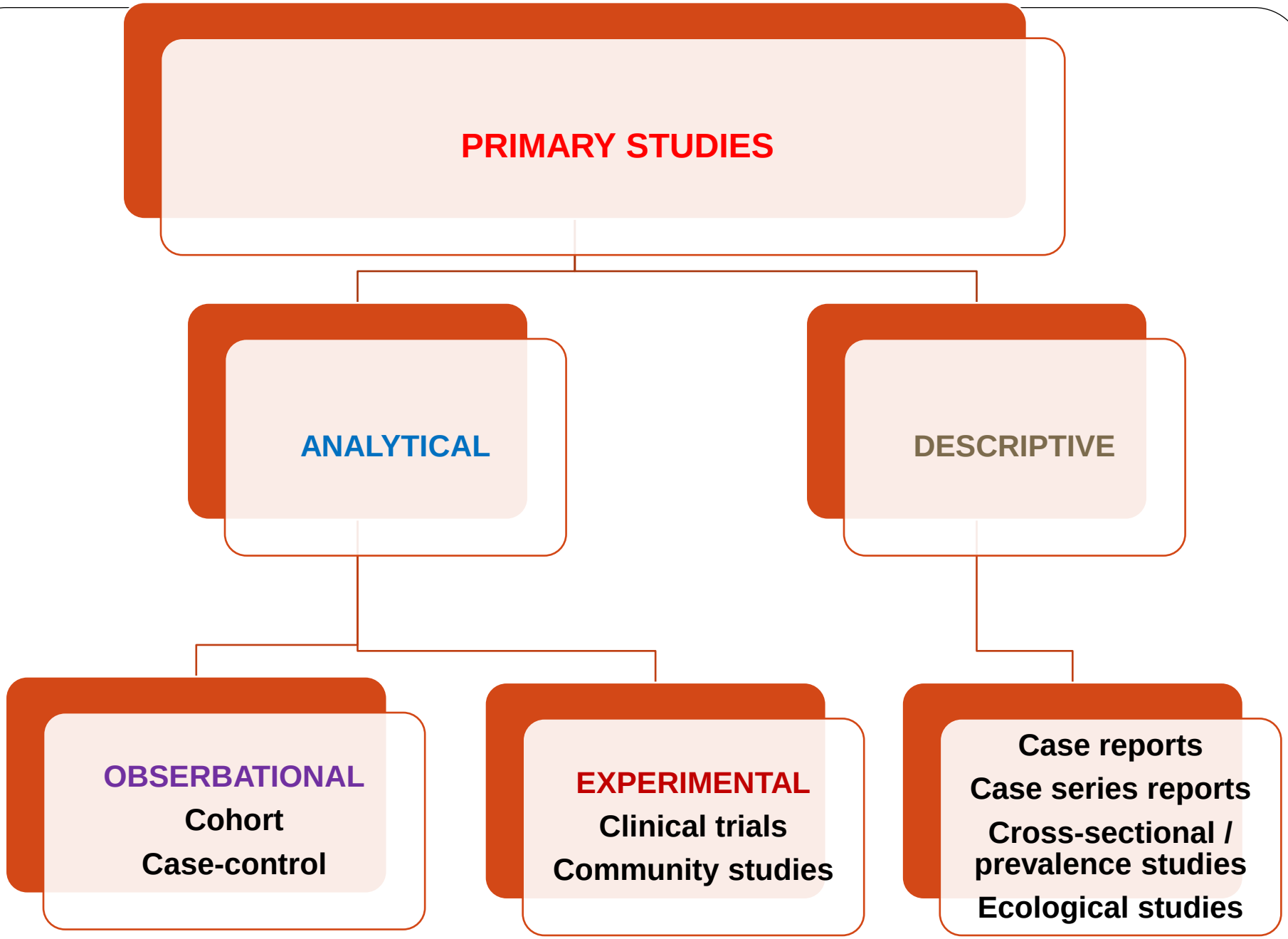
Topics

1. **Experimental Studies: essence, objectives, types**
2. **Clinical Epidemiology, importance, objectives**
3. **Randomized clinical trials/ randomized controlled trials (RCT): stages, phases and types**
4. **Concepts of randomization and blinding**
5. **Estimating the effect in RCT. Advantages and disadvantages of RCT**
6. **Diagnostic studies. Screening tests, types of screening.**
7. **Sensitivity, Specificity, Predictive values and Probability ratios in diagnostic studies.**


PRIMARY STUDIES
ANALYTICAL
DESCRIPTIVE
OBSERBATIONAL
Cohort
Case-control
EXPERIMENTAL
Clinical trials
Community studies
Case reports
Case series reports
Cross-sectional / prevalence studies
Ecological studies

EXPERIMENTAL STUDIES

- **All previous studies were passive**
- **In experimental studies the researcher assign the Exposure (treatment or intervention)**

EXPERIMENTAL STUDIES

- **Experimental (intervention) studies are distinguished by the design that is dedicated to evaluating the effect of treatment or public health practices in well-defined populations.**

EXPERIMENTAL STUDIES

Are used for

- **Testing of new drugs / methods of treatment**
- **Testing of new vaccines**
- **Evaluation of prevention strategies**
- **Testing of health educational methods**

EXPERIMENTAL STUDIES

Types

- **Therapeutic studies (treatment)**
- **Preventive studies (prevention)**

CLINICAL EPIDEMIOLOGY

- **Generates new tactics and strategies for diagnosis and treatment**
- **Identifies and solves diagnostic and therapeutic problems**

CLINICAL EPIDEMIOLOGY

“ ... represents the use of epidemiological methods by clinicians”

(D.Sacket,1969)

CLINICAL EPIDEMIOLOGY

“Discipline that addresses the creators of scientific studies, researchers, teaching them the methodology to be applied so that the results of the study to be valid”

(C.Baicuş,2002)

CLINICAL EPIDEMIOLOGY

OBJECTIVES (1)

1. **Data collection, measurement, classification**
2. **Risk evaluation**
3. **Identifying individuals with particularities**
4. **Search of causal associations**

CLINICAL EPIDEMIOLOGY

OBJECTIVES (2)

5. Study of the best therapeutic intervention
6. Evaluating the effectiveness of treatments or care methods
7. Establishing the prognosis according to the therapeutic result obtained

RANDOMIZED CLINICAL STUDIES/ RANDOMIZED CONTROLLED TRIALS (RCT)

- **RCT is a controlled experiment used to evaluate the safety and efficiency of treatments applied to diseases and health problems**

- **RCT is essential for the process of developing and accepting new treatments**

RANDOMIZED CLINICAL STUDIES(RCT)

- **The first therapeutic studies were studies without a control group, which were based on the evolution of the disease before and after the administration of the studied medication (comparative studies).**
- **The testing of drugs used before the randomize clinical studies was based on a series of cases**

RANDOMIZED CLINICAL STUDIES

stage I – preclinical studies

stage II - clinical trials :

initial evaluation of volunteers

evaluation of treatment effectiveness (100-200 persons)

- **- evaluation of the new treatment in a larger number of volunteers (500-1500 persons)**
- **- study of long-term effects of the treatment**

STAGE I - PRECLINICAL STUDIES

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RCT: stage I

- **Laboratory experiments**
  - **in vitro**
  - **on animals**
- **Objective: Preclinical studies provide pharmacological and toxicological information necessary for the preparation of human studies**

RCT: stage I

***3 „R”* concept (Russel and Burch, 1959)**

- **Reducing the number of experiments on animals**
- **Reducing the number of animals in scientific experiments**
- **Respect the ethics**

RCT: stage I

***additional to* + *3 „R”* concept**

- **Use of alternative methods, which exclude the use of animals**

- **Ensuring the highest level of protection and welfare of experimental animals**

STAGE II – CLINICAL TRIALS

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STUDY INCLUSION CRITERIA

- **Demographic characteristics (age, sex,…)**
- **Geographical and temporal criteria (population needs to be available; eg. if patients live in a hard-to-reach area they will not be able to come to study visits)**
- **Clinical characteristics (all patients must have the disease that we expect the study medication to improve / cure)**

STUDY EXCLUSION CRITERIA

- **Exclusion of persons who may compromise the quality of the data or the interpretation of the results (alcoholics, patients with psychiatric problems, subjects likely to go to another region, etc.)**

- **Ethical reasons (pregnancy, minors)**

- **Volunteers who did not sign the "Informed Consent" ("Participation Agreement“)**

If someone decided to take part in a clinical trial, can change the mind?
(1)

- **Participation in a clinical trial is a voluntary, optional act**
- **In order to make an informed decision, the doctor gives the participant the necessary information in written and verbal form: the objectives of the study, the risks, etc.**
- **The volunteer has time to think about making the decision whether to participate or not (Careful analysis of medical information and the conditions under which consent is given)**

If someone decided to take part in a clinical trial, can change the mind?

- **No investigation or treatment related to a clinical trial may be performed without the written consent of the patient**
- **The volunteer is free to refuse to participate in such a study. The refusal does not affect the treatment of the patient in the hospital**
- **The volunteer may withdraw from participation in the clinical trial at any time without affecting the way he or she will be treated and cared for in the hospital.**

RCT: stage II phase 1

Initial evaluation of human participants (screening)

- **Informed agreement**
- **Treatment administration (20 – 100 volunteers)**
- **Objective: evaluation of treatment safety and tolerance**

SCR: stage II phase 2

Evaluation of treatment effectiveness

- **100 – 200 participants**
- **Objectives:**

-Evaluation of the potential effectiveness of the treatment

-Assessment of the optimal method / dose of treatment administration

RCT: stage II phase 3

Evaluation of the new treatment

- **500 – 1500 participants**
- **Objectives:**

  - Evaluating the effectiveness of the new treatment

  - Collection of additional information concerning treatment safety

RCT: stage II phase 4

Study the long-term effects of treatment (Post-marketing)

- **The treatment is approved for use**
- **Objectives: study of the long-term effects of treatment**

RCT

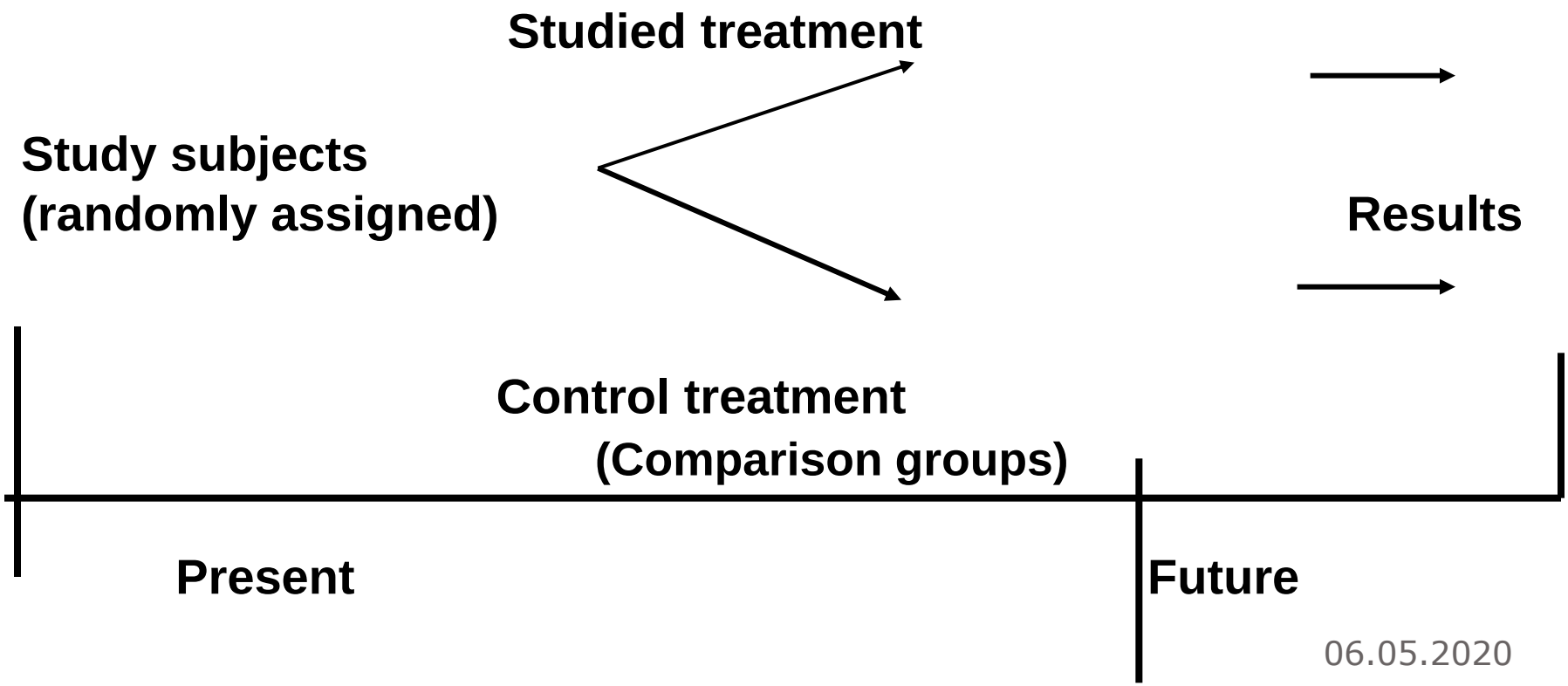
- **Most studies present comparative clinical trials, in which the comparison groups are presented by the new treatment group and the control / placebo treatment group.**

RCT: types

- **Parallel treatment type**
- **Successive / Sequential treatment type**

RANDOMIZED CONTROLLED TRIALS

Parallel treatment type



RCT: types

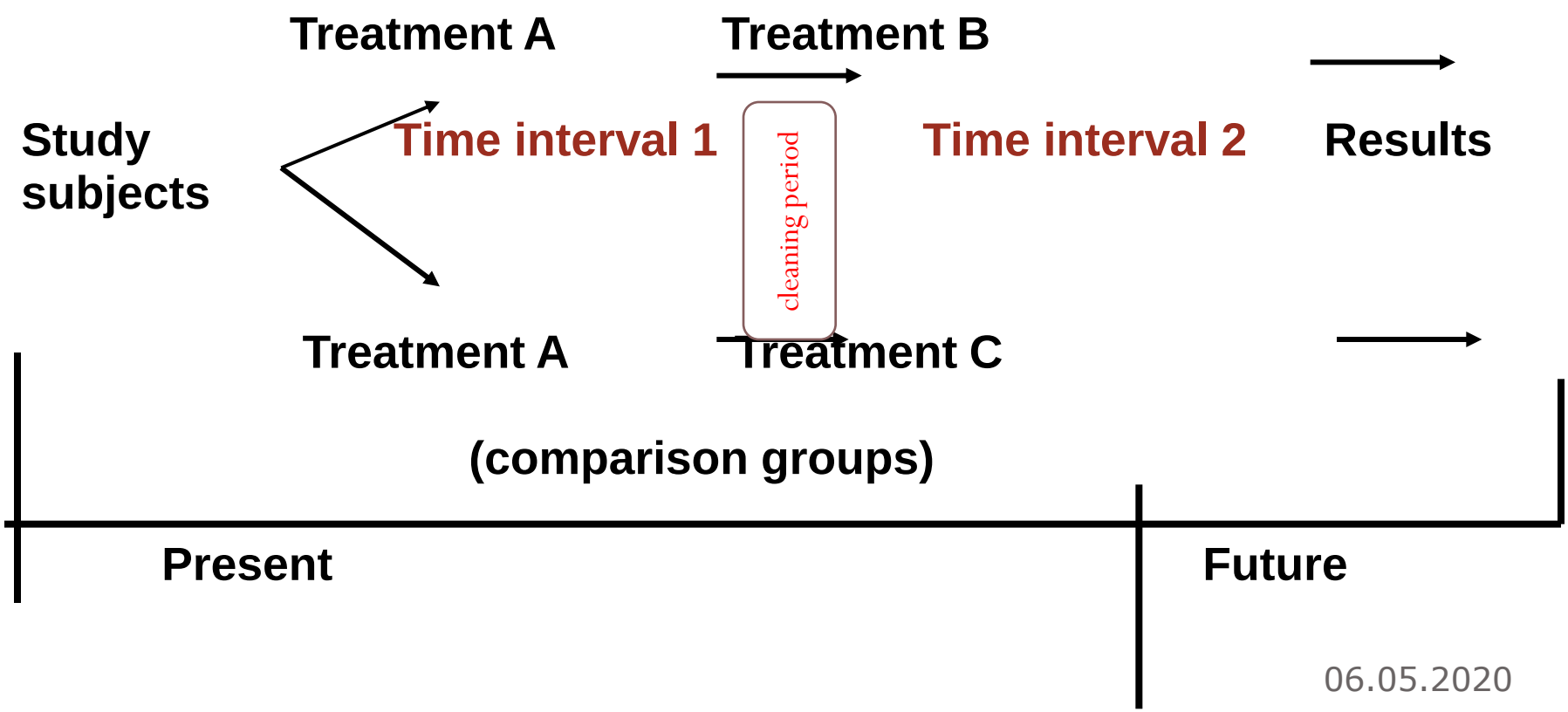
- **Successive treatment type**

  - ***Substitution treatment type***

  - ***The crossover***

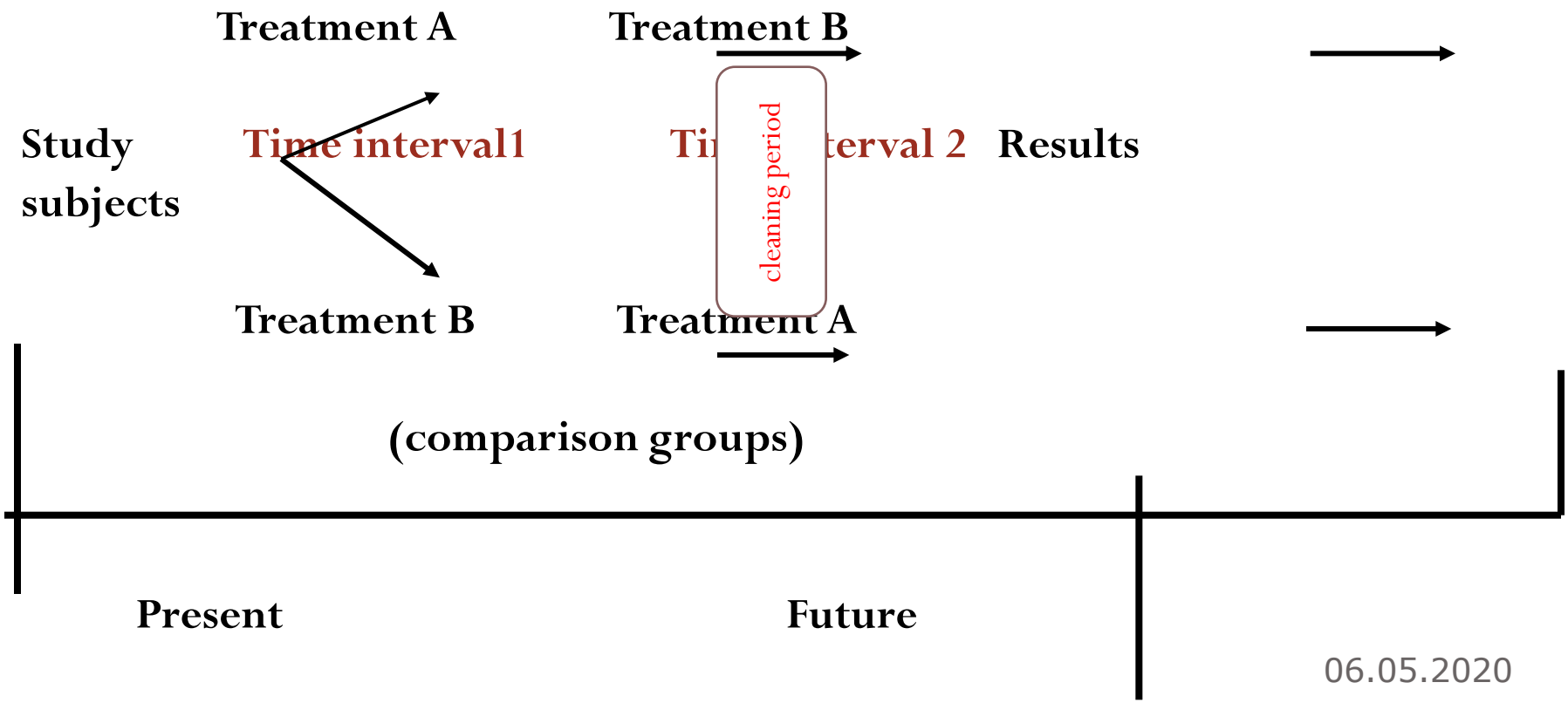
RANDOMIZED CONTROLLED TRIALS

Substitution treatment type



RANDOMIZED CONTROLLED TRIALS

The crossover



CLEANING/WASHING PERIOD

- **The period in which another treatment is started before the second treatment**

- **Sometimes - at the beginning of the SCR - if participants used other treatments before being included in the research**

Randomization
Hidden allocation

Randomization

- **Procedure that ensures the random allocation of patients in the experimental group and the control group**

Hidden allocation (randomized)

Randomization

- **Ensures no differences between patient groups**
- **Decreases the probability of systematic error (occurs when samples are not homogeneous according to one criterion or another)**

Hidden allocation (randomized)

- **Every individual who enters in a study has the same chance of receiving one of the possible treatments**

- **The beauty of randomization is that it ensures (if the sample is large!) the equal distribution between the groups with treatment and control of both known and unknown determinants.**

Hidden allocation (randomized)

- **The randomization process must be performed by someone who is not responsible for recruitment**

- **What are the appropriate randomization methods?**

ADEQUATE RANDOMIZATION METHODS

- **Centralized randomization schemes**
- **Randomized schemes controlled by a pharmacy**
- **Numbered or coded containers**
- **Computerized schemes**
- **Numbered / colored envelopes**

“THE BLINDING”

- An RCT may be blinded, (also called "masked") by "procedures that prevent study participants, caregivers, or outcome assessors from knowing which intervention was received

”THE BLINDNESS”

- **It is done using placebo**
- **Placebo consists of pills / injections / therapeutic techniques identical in appearance, taste, etc. with medication, but not containing active medication**
- **The blindness is essential especially when the effects can be judged subjectively**

“THE BLINDNESS”

- **CLASSIFICATION**

- “single-blind",
- “double-blind",
- “triple-blind";
- “Four - blind”

“THE BLINDNESS”

- **Types: randomized**
  - **Randomized single - blind trial:**
    - **Medicine A vs medicine B**
    - **Medicine vs placebo**
      - **The patient doesn’t know if medicine A vs B / medicament vs placebo is being administered**

“BLINDING”

- Types: randomized
  - Randomized double - blind trial
    - Medicine A vs medicine B
    - Medicine vs placebo
      - Neither the patient nor the researcher knows whether A drug vs B drug is being given / or medicine vs placebo

“BLINDING”

- **Types: randomized**
  - **Randomized triple - blind trial**
    - **Medicine A vs medicine B**
    - **Medicine vs placebo**

      - **Neither the patient nor the researcher and statistician know whether A drug vs B drug is being given / or medicine vs placebo**

“BLINDING”

- **Types: randomized**
  - **Randomized four- blind trial**
    - **Medicine A vs medicine B**
    - **Medicine vs placebo**
      - **Neither the patient nor the researcher, statistician and laboratory staff know whether A drug vs B drug is being given / or medicine vs placebo**

SERIAL TRIAL

- **Each patient is also his own control**
- **Applicable in chronic diseases**
- **It is administered successively, in equal periods, with pause periods:**
  - **Medicine A therefore medicine B**
  - **Medicine therefore placebo**
- **The therapeutic efficacy is compared at the two periods**

Advantages: homogeneity

QUASIRANDOMIZED ALLOCATION

- **An allocation method that is not really random**
- **Ex .: Allocation based on date of birth, day of the week, no. observation sheet**

VALIDITY: Is "blindness" important?

- **Knowing the allocation to treatment by the clinician, patient or researcher can be detrimental**

- **In 250 trials, in which the allocation to treatment was not "blind", the result was overestimated by 41.0%**
  - Schultz et al. JAMA 1995;273:408-12

CONTROLLED THERAPEUTIC STUDY

- **Refers to a study comparing one or more samples operated with one or more control samples**

- **A controlled therapeutic study may not be randomized, but any randomized clinical trial is controlled**

PATIENT TRACKING (“FOLLOW-UP”)

- **All patients included in the study must participate in the final statistics**
- **Ideally, “lost to follow-up” should represent less than 5% of the included patients so as not to affect the final results of the study**
- **"Lost to follow-up" patients have a different prognosis than those who reach the end of the study, regardless of the group in which they were assigned**
- **When "lost to follow-up" are over 20%, the study can no longer be considered valid**

CLINICAL STUDIES IN THE POPULATION

- **It involves people who show no signs of illness but are at risk**
- **Data are collected from the general population, not from the target population**
- **Purpose: to prevent the occurrence of diseases**

CLINICAL STUDIES IN THE POPULATION

Examples

- **Salk vaccine test for polio prevention**
- **Prevention of coronary heart disease in mature adults**
- **Testing protection methods vs. the effect of pesticides**
- **Evaluation of the impact of the elimination of Pb-based paints from the domestic environment on children's lead**

COMMUNITY CLINICAL STUDIES

- **Community clinical trials target groups formed in communities**
- **They are recommended for diseases determined / influenced by the social conditions of the respective community**

COMMUNITY CLINICAL STUDIES

Example

- **The impact of an education program in 2 high-risk populations on reducing the incidence and mortality of CVD (individual and general counseling, through the media on reducing fat intake, etc.).**
- **The results were better in the population to which individual methods were applied.**

ESTIMATING THE EFFECT

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Contingency table 2x2

	Present effect	Absent effect	Total
"New" treatment	**a**	**b**	**a+b**
"Control" treatment	**c**	**d**	**c+d**
Total	**a+c**	**b+d**	**a+b+c+d**

RATE (RISK) OF THE EVENT IN THE EXPERIMENTAL GROUP

- ***REE*** **- the proportion of patients in the experimental group in which the event occurred**

- ***REE=a/(a+b)***

RATE (RISK) OF THE EVENT IN THE CONTROL GROUP

- ***REC*** **- the proportion of patients in the control group in which the event occurred**

- ***REC=c/(c+d)***

RELATIVE RISK

- ***RR*** **- the risk of the event in the experimental group compared to the risk of the event in the control group**

- ***RR= REE/REC***

- ***RR= a:(a+b)/c:(c+d)***

RELATIVE RISK REDUCTION (INCREASE)

- ***RRR*** **- proportional reduction of the negative (positive) event rate between the experimental group and the control group**

- ***RRR=(REE-REC) /REC or***

- ***RRR=1-RR***

ABSOLUTE RISK REDUCTION (INCREASE)

- ***ARR*** **- the absolute difference between the rates of the negative (positive) event in the experimental group and the control group**

- ***ARR=| REE – REC |***

EVEN PROBABILITY

- ***E P*** **- the ratio of the number of people supporting the event to the number of people not supporting the event**

PROBABILITY OF THE EVENT IN THE EXPERIMENTAL GROUP

- ***PEE*** **- the ratio of the number of people in the experimental group that supports the event to the number of people in this group that does not support the event**

- **PEE=a/b**

PROBABILITY OF THE EVENT IN THE CONTROL GROUP

- ***PEC*** **- the ratio of the number of people in the control group who support the event to the number of people in this group who do not support the event**

- **PEC=c/d**

RELATIVE PROBABILITY OF THE EVENT

- ***RP*** **- is a measure of the clinical effectiveness of treatment**
- ***PRE=PEE/PEC***
- **PRE= a/b : c/d = a*d / b*c**

NUMBER NEEDED TO TREATE

- **Number needed to treat to prevent a case of adverse outcome (for the occurrence of an adverse event)**

- ***NNT=1/ARR***

NNT CONCEPT

- **The most important tool for assessing the magnitude of the effect of an internationally accepted treatment**

- **The example below shows the evaluation of 2 drugs A and B for the same disease in 2 different studies (medication vs placebo)**

Example: **A/X Treatment**

	Results		
	YES	**NO**	**TOTAL**
Experimental group (+)	5 a	95 b	100 m_1
Control group (-)	10 c	90 d	100 m_0
TOTAL	15 n_1	185 n_0	200 t

A/X Treatment

- R treated=5/100=5%
- R untreated =10/100=10%
- RR=R treated /R untreated =5/10=0.5
- RRR=1-0.5=0.5
- ARR=R untreated -R treated = (10/100-5/100)=5/100=10-5=5%
- NNT=1/ARR=20

Example: B/X Treatment

	Results		
	YES	NO	TOTAL
Experimental group (+)	20 a	80 b	100 m_1
Control group (-)	40 c	60 d	100 m_0
TOTAL	60 n_1	140 n_0	200 t

B/X Treatment

- R treated =20/100 =20%
- R untreated =40/100 = 40%
- RR=R treated /R untreated =20/40=0.5
- RRR=1-RR=0.5
- ARR=R untreated -R treated =

40/100-20/100=20/100=20%

- NNT=1/ARR=5

RESULTS

A/X Treatment

- R treated =5%
- R untreated =10%
- RR=0.5
- RRR=0.5
- ARR=5%
- NNT=20

B/X Treatment

- R treated =20%
- R untreated = 40%
- RR=0.5
- RRR=0.5
- ARR=20%
- NNT=5

DETERMINING OF CI

- $CI = RR^{(1 \pm z / x)}$

$$x^2 = \frac{(t-1)[(a \times d) - (b \times c)]^2}{n_1 \times n_0 \times m_1 \times m_0}$$

- For 95% veracity , probability, z = 1,96

$$CI_{sup.\ lim.} = RR^{(1 + z / x)}$$
$$CI_{inf.\ lim.} = RR^{(1 - z / x)}$$

EVALUATION OF RESULTS

RR/RP	Result
0.0 – 0.3	Strong protection factor
0.4 – 0.5	Moderate protection factor
0.6 – 0.9	Low protection factor
1.0 - 1.1	Indifferent factor
1.2 – 1.6	Low risk
1.7 – 2.5	Moderate risk
>2.5	Very high risk

RELATIVE RISK

- **Interpretation of the RR value**
  - **Result (complication) is RR times more frequent in the experimental group than in the control group**
  - **RR values close to 1 indicate no link between the new treatment and the desired outcome**
  - **Subunit values indicate a positive association between new treatment and desired outcome (protection factor)**

RR INTERPRETATION BASED ON CI

- **For RR with a value greater than 1 and CI with values close to the calculated RR that does not include the value 1 we can decide that there is a negative association between the new treatment and the desired result**

- **For RR values greater than 1 but CI that includes the value 1 it can be concluded that the association between new treatment and follow-up result is indifferent**

RR INTERPRETATION BASED ON CI

- **For RR with a value less than 1 and CI with values close to the calculated RR that does not include the value 1 we can decide that there is a positive association between the new treatment and the desired result (it is a protective factor)**

- **For RR values less than 1 but CI that includes the value 1 it can be concluded that the association between new treatment and follow-up result is indifferent**

Table 1. Risk of complications in the group of patients with the modified method of treatment / traditional method

Characteristic	L_1 n=36	L_0n=36	RR	95.0%CI
Subluxations	3	4	0,75	0,181 – 3.115
Ossification	1	8	0,13	0,016 – 0,949
Arthrosis	3	9	0,33	0,098 – 1,132
Implant degradation	2	11	0,18	0,043 – 0,763
Polytrauma	1	2	0,50	0,047 – 5,278
Without complications	28	9	3,11	1,721 – 5,624

Table 2. Efficacy of treatment in the group of patients with the modified method of treatment / traditional method

Characteristic	L_1 n=36	L_0n=36	PR	95.0%CI
Subluxations	3	4	0.73	0.152-3.510
Ossification	1	8	0.10	0.012-0.848
Arthrosis	3	9	0.27	0.670-1.108
Implant degradation	2	11	0.13	0.027-0.657
Polytrauma	1	2	0.49	0.042-5.608
Without complications	28	9	10.5	0.533-31.207

Table 3. Risk of complications in the research group compared to the control group

	L1 (n_1=65)	L0 (n_0=81)	RR	CI95.0%	p	NNT
After 6 months of treatment	13	1	16,2	2,1758-120,6196	0,0065	5,3
After 12 months of treatment	0	2	0,25	0,0121-5,6870	0,3660	43,6
After 24 months of treatment	1	4	3,42	1,1652-10,0310	0,0252	8,4
After 36 months of treatment	1	1	1,22	0,0795-19,5434	0,8755	329,0

When are RCTs not required?

- **When an obviously useful treatment is known**
- **When a previous study or meta-analysis has already demonstrated the effect**

When are RCTs not practical?

- **When it is unethical to perform randomization or ask for consent at randomization**

- **When demonstrating the effect of a treatment requires a very large number of patients**

ADVANTAGES OF RCT

- **Random selection is the only one known effective method to control selection error**
- **A RCT allows the standardization of eligibility criteria in the evaluation of results**
- **An RCT has simultaneous comparison groups: any external intervention is unlikely to influence the results because it will affect both groups equally.**

DISADVANTAGES OF SCR

- **RCTs are expensive**
- **RCTs may be subject to a lack of representativeness: volunteers may differ from the general population**
- **An RCT can be open to challenges: is it ethical not to treat a group?**

DIAGNOSTIC STUDIES

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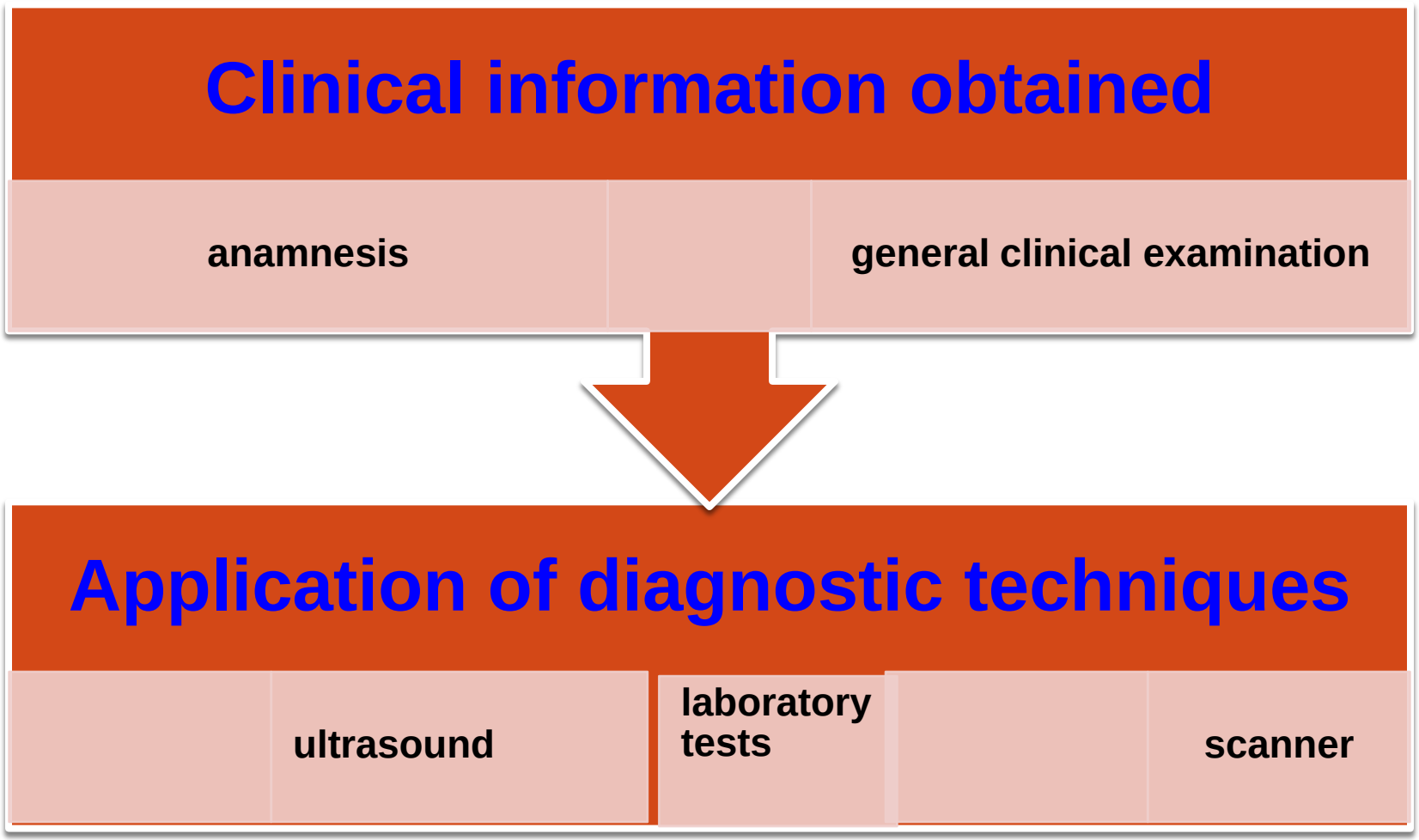
DIAGNOSTIC STRATEGY



- **The effectiveness of a test refers to its technical capabilities and accuracy**
- **The validity of a test means its ability to identify subjects "affected" by the disease and healthy subjects**

SCREENING TESTS

- **Screening is almost always secondary prevention**
- **Screening is applied to asymptomatic and / or apparently healthy people**
- **Screening should be easily acceptable, possible and available**

CHARACTERISTICS OF SCREENING TESTS

- **Economically rational**
- **Convenient**
- **Risk and discomfort free**
- **Acceptable for a large number of individuals**
- **Valid and safe**

TYPES OF SCREENING

- **SIMPLE – for an illness (TB)**
- **MULTIPLE – includes several independent tests**
- **MULTIPHASE - several methods / steps (ELISA test)**
- **SISTEMATIC – for mass detection**

Table
2 X 2

		Disease	
		Present	Absent
Test	Positive	Positive true **a**	Positive false **b**
	Negative	Negative false **c**	Negative true **d**

WHAT ARE THE RESULTS?

- **Sensitivity**
- **Specificity**
- **Predictive values (positive, negative)**
- **Probability ratio (positive, negative)**

SENSITIVITY

- **Probability of positive test in patients affected by disease**

- ***A very high sensitivity, in the conditions of the negative test, excludes the presence of the disease***

Senstivity = a / (a + c)

- **True positive rate =**

  PT / (PT + NF)
- **Sensibility = TPR =**

  9 / (9+6) = 9/15 = 60%
- **Rate of people with the disease who tested positive for this test**
- **False negative rate =**

  1 – sensibility = 40%

	D	ND	
+	9	5	14
-	6	20	26
	15	25	40

SPECIFICITY

- **Probability of negative test in patients without the disease**

- **A very specific test (with high specificity), when is positive, confirms the presence of the disease**

Specificity= d / (b + d)

- **True negative rate =**

  NT / (PF + NT)
- **Specificity = TNR =**

  20 / (5+20) = 20/25 = 80%
- **Rate of people without disease who tested negative to this test**
- **False positive rate =**

  1 – specificity = 20%

	D	ND	
+	9	5	14
-	6	20	26
	15	25	40

Positive predictive value = a / (a + b)

- **PPV = PT / (PT + PF)**
- **PPV = 9 / (9+5) = 9/14 = 64%**
- **Percentage tested positive that are correctly classified as with disease**

	D	ND	
+	9	5	14
-	6	20	26
	15	25	40

Negative predictive value = d / (c + d)

- **NPV = NT / (NT + NF)**
- **NPV = 20 / (6+20) = 20/26 = 77%**
- **The percentage of negative tests that are correctly classified as NOT having the disease**

	D	ND	
+	9	5	14
-	6	20	26
	15	25	40

PROBABILITY REPORTS

- **Positive Probability Ratio (PR +) - how many times the probability of the disease increases in the case of a positive test**

- **PR + = probability of disease in an individual with a positive test / probability of no disease in an individual with a positive test**

PROBABILITY RATIO

- **Negative Probability Ratio (RP-) - how many times the probability of the disease decreases with the negative test**

- **RP- = probability of disease in an individual with a negative test / probability of missing the disease in an individual with a negative test**

PROBABILITY RATIO

- **PR + = sensitivity / (1-specificity)**

- **PR- = (1-sensitivity) / specificity**

PR INTERPRETATION

RP	INTERPRETATION
>10	A large increase in the probability of the disease
5 - 10	A moderate increase in the likelihood of the disease
2 – 4.9	A small increase in the probability of the disease
1.1 – 1.9	A minimal increase in the probability of the disease
1.0	The probability of the disease does not change
0.5 – 0.9	A minimal decrease in the probability of the disease
0.3 – 0.4	A small decrease in the probability of the disease
0.1 – 0.2	A moderate decrease in the probability of the disease
<0.1	A large decrease in the probability of the disease

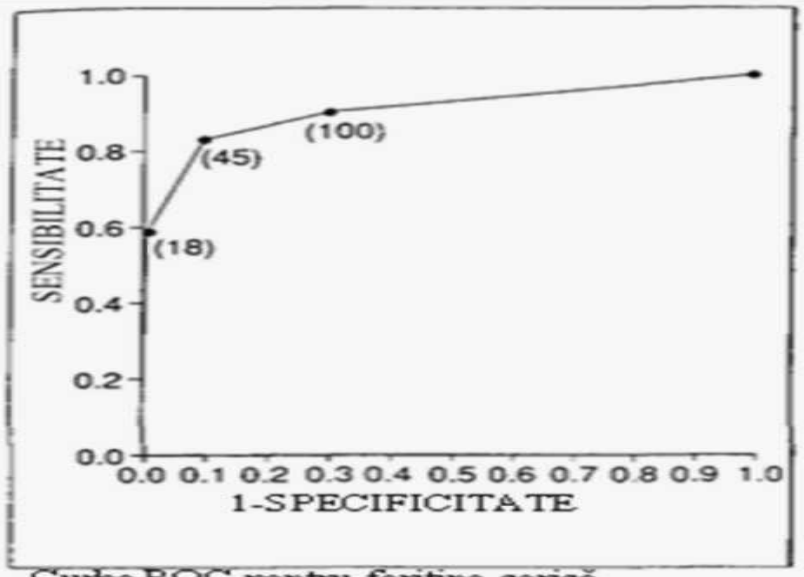
ROC (Receiver Operating Characteristic) curve

- **Graphic of evaluating the ability of a diagnostic test to differentiate healthy subjects from sick subjects**

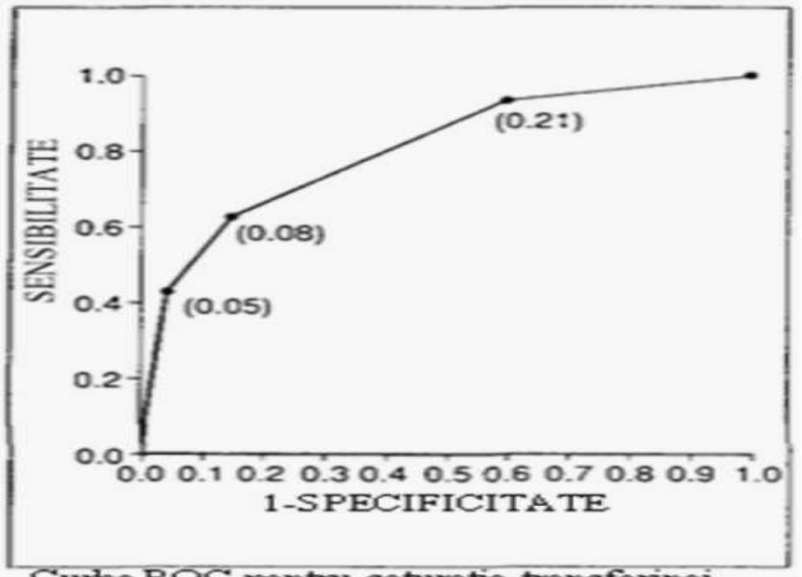
- **It is an experimental curve of sensitivity variations, on the ordinate, depending on those of the false positive rate (1-specificity), on the abscissa**

ROC (Receiver Operating Characteristic) curve

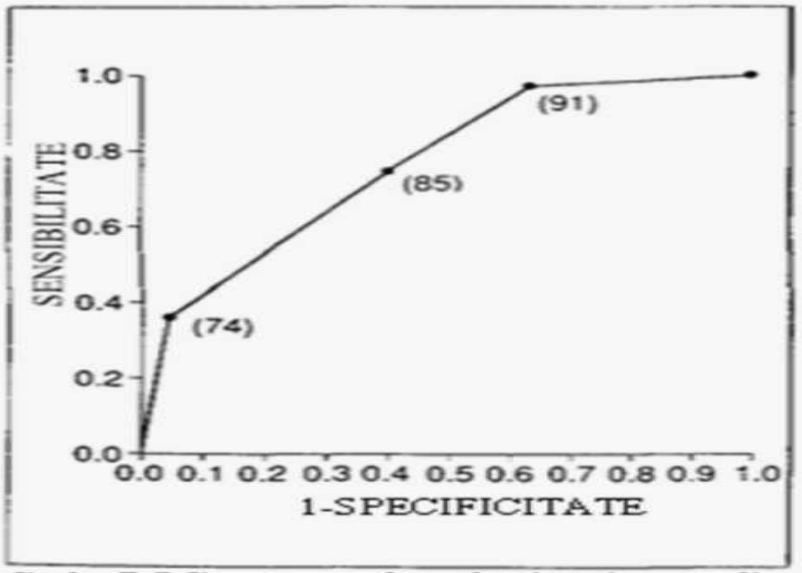
- **The ROC curve provides a rational analysis of the different possible threshold values**
- **The ROC curve allows the comparison of multiple threshold values and the choice of the optimal value**
- **It is constructed by calculating for each threshold value considered, sensitivity and specificity.**



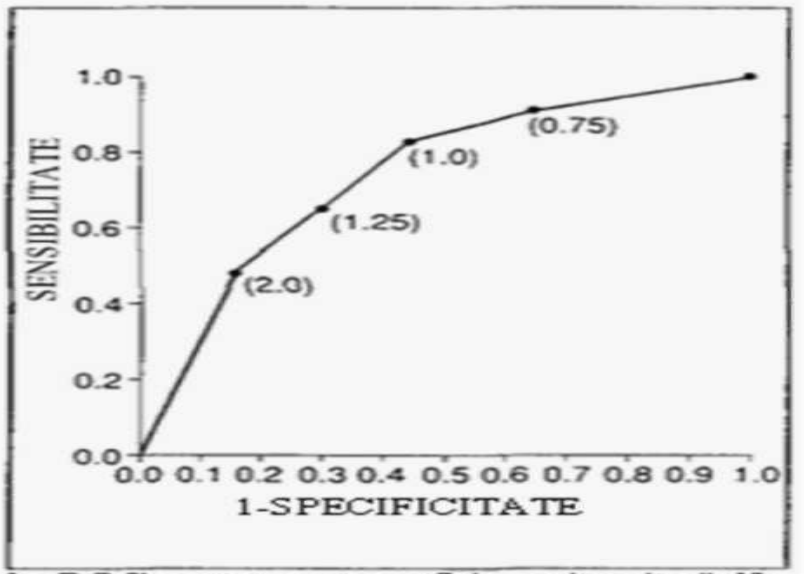
Curba ROC pentru feritina serică



Curba ROC pentru saturația transferinei



Curba ROC pentru volumul eritrocitar mediu



Curba ROC pentru protoporfirina eritrocitară liberă