Reference Values

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Introduction

- Medicine is an art and a science in the service of human (Patients and healthy individuals)
- To improve the health of their patients, physicians;
  - collect empirical data,
  - interpret these data using scientific knowledge and professional experience,
  - make decisions concerning diagnoses,
  - recommend preventive measures, and
  - execute therapeutic actions.

Introduction

- Data collected during the medical interview, clinical examination, and supplementary investigations must be interpreted by comparison with reference data
- The physician does this when making a diagnosis.

Introduction

- The interpretation of medical laboratory data is an example of decision making by comparison.
- Therefore, reference values are needed for all tests performed in the clinical laboratory, not only from healthy individuals but from patients with relevant diseases.

Introduction

- An observed value in an individual should be related to relevant collections of reference values, such as values from healthy persons, from the undifferentiated hospital population, from persons with typical diseases, and from ambulatory individuals, along with previous values from the same subject.
- A patient's laboratory result simply is not medically useful if appropriate data for comparison are lacking.

Definition

- Term *normal* (commonly used) is obsolete
- Use of the term *reference values* is recommended. Other terms are: *reference individual*, *reference limit*, *reference interval* and *observed values.*
- Reference values are results of a certain type of quantity obtained from a single individual or group of individual corresponding to a stated description, which must be spelled out and made available for use by others (IFCC).

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Definition

- Qualifiers associated with the term reference values such as*: health-associated reference values*, *diabetic patient*, *hospitalized diabetic patient*, *ambulatory diabetic patient*.
- Reference values are not related with health.

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Types of Reference Values

- The reference values can be: *subject-based* or *population-based*
- Subject-based are reference values are previous values from the same individual, obtained when the individual was in defined state of health
- Population-based are those obtained from a group of systematically defined reference individuals.

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Establishment of Reference Values

Certain conditions are mandatory to make the comparison of a patient's laboratory results with reference values possible and valid:

- All groups of reference individual should be clearly defined
- The patient examined should resemble sufficiently the reference individuals (in all groups selected for comparison) in all respects other than those under investigation

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Establishment of Reference Values

- The conditions under which the samples were obtained and processed for analysis should be known
- All quantities compared should be of the same type
- All lab results should be produced with use of adequately standardized methods under sufficient analytical quality control

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Establishment of Reference Values

- The stages in the pathogenesis of the diseases that are the objective for diagnosis should be stated
- The diagnostic sensitivity and specificity, prevalence, and clinical costs of misclassification should be known for all lab tests used

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Selection of Reference Individuals

- A set of selection criteria determines which individual should be included
- These includes:
  - Statements describing source of population
  - Specification criteria for health or disease of interest
- Selection is based essentially on the application of defined criteria to a group of examined candidates

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Table 1: Examples of Exclusion Criteria for Health-associated Reference Values

DISEASES	
Risk Factors	
	Obesity
	Hypertension
	Risk from occupation or environment
	Genetically determined risks
Intake of Pharmacologically Active Agents	
	Drug treatment for disease or suffering
	Oral contraceptives
	Drug abuse
	Alcohol
	Tobacco

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Table 1: Examples of Exclusion Criteria for Health-associated Reference Values

Specific Physiological States	
	Pregnancy
	Stress
	Excessive exercise

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Selection of Reference Individuals

- Separate reference values for sex, age group, and other criteria are necessary
- Describe partition criteria for the sub classification of the set of selected reference individuals into more homogeneous groups (table 2)
- Small number of partition criteria to get sufficient samples

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Selection of Reference Individuals

- Age and sex: Several analytes vary significantly among different age and gender groups
- Age can be categorised by equal intervals or used qualitatively: postnatal, infancy, childhood, pre-pubertal, pubertal, adult, pre-menopausal, menopausal or geriatric
- Height and weight for categorizing children

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Table 2: Partitioning Criteria for Sub-grouping

AGE	Not necessarily categorized by equal intervals
SEX	
GENETIC FACTORS	
	Ethnic origin
	Blood groups
	Histocompatibility antigens (HLA)
	Genes
PHYSIOLOGICAL FACTORS	
	Stage in menstrual cycle
	Stage in pregnancy
	Physical condition
OTHER FACTORS	
	Socio-economic
	Environmental
	Chronobiological

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Sample Collection

- Pre-analytical standardization of the
  - (i) preparation of individuals before sample collection,
  - (ii) sample collection activity,
  - (iii) handling the sample before analysis may minimize the bias or variation
- Magnitude of pre-analytical sources of variation clearly are not equal for different analytes:

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Sample Collection

- Body posture during sample collection is highly relevant for the establishment of reference ranges for non-diffusional analytes e.g. albumin in serum but irrelevant for diffusional ones
- Ingestion of drugs before sample collection presents a special problem. Drugs should taken 2 days before sample collection
- Use of indispensable drugs such as contraceptive pills, essential medication may be a criterion for exclusion or partition

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Sample Collection

The total testing process The process phases	Responsibility	Action
Preanalytical phase	Central laboratory#, All participating laboratories*	1. Organization of the study Establishing the inclusion and exclusion criteria Informing each laboratory of the sample size Deciding the essential items in the questionnaire and distributing to all participating laboratories Communicating with the participating laboratories and informing about the procedures (e.g. blood collection and preparation of the serum samples) to standardize the pre-analytical phase in each laboratory #

Sample Collection

The total testing process The process phases	Responsibility	Action
Preanalytical phase	Central laboratory#, All participating laboratories*	2. Using the same protocol in each laboratory (e.g. selection and preparation of the volunteers, blood collection and sample preparation) #,*

Process

The total testing process The process phases	Responsibility	Action
Preanalytical phase	Central laboratory#, All participating laboratories*	3. Transportation of the samples to central laboratory * 4. Storing of the remaining samples for cross-check study in each laboratory*
Analytical phase	Central laboratory#, All participating laboratories*	1. Using a reference measurement system, certificated reference materials/value assigned sera, standardization of the assays # 2. Quality control of the assays #,* 3. Analyzing all the samples # 4. Analyzing the cross-check samples *

Data Treatment

The total testing process The process phases	Responsibility	Action
Postanalytical phase	Central laboratory#, All participating laboratories*	1. Reporting of the test results to each laboratory # 2. Data analysis and derivation of the RIs (common RIs, if it's possible) # 3. Reporting of cross-check results and the RIs for each of the local laboratory # 4. Using the calculated RIs #,

Statistical Treatment of Reference

- After specimen analysis, reference range are subjected to statistical treatment:
  - Partitioning
  - Inspection of distribution group
  - Identification of outliers
  - Determination of reference limits

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Non-parametric methods

- Non-parametric methods make no assumptions about the distribution of the data
- There are non-parametric methods for characterizing data, as well as for comparing data sets
- These methods are also called *distribution-free, robust,* or sometimes *non-metric* tests

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Parametric Methods

- Assumes certain types of distribution and is based on estimates of population parameters: mean, SD
- E.g. parametric method is used if the true distribution is believed to be gaussian and reference limits are determined as the values located two SD below and above the mean
- Based on gaussian distribution

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Application to Reference Ranges

- Reference ranges for normal, healthy populations are customarily defined as the "central 95%".
- An entirely non-parametric way of expressing this is to eliminate the upper and lower 2.5% of data, and use the remaining upper and lower values to define the range.

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- *Inspection Method:* Verify reference populations are equivalent
- *Limited Validation:* Collect 20 reference specimens
  - *No more than 2 exceed range*
  - *Repeat if failed*
- *Extended Validation:* Collect 60 reference specimens; compare ranges.

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Method Sensitivity

- The *analytical* sensitivity of a method refers to the lowest concentration of analyte that can be reliably detected.
- The most common definition of sensitivity is the analyte concentration that will result in a signal two or three standard deviations above background.

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Biological Factors Affecting the Interpretation of Results

- *Sex of patient*-serum creatinine
- *Age of the patient*-neonates, children, adults, elderly
- *Effect of diet*-results differ: fasting or after meal
- *Time when sample is taken*-day and night variations
- *Stress and anxiety*-may affect analyte of interest
- *Drug history*-specific effects on plasma conc.

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Biological Factors Affecting the Interpretation of Results

- *Posture of the patient*-redistribution of the fluid
- *Effects of exercise*-strenous exercises can release enzymes from tissues
- *Medical history*-infection, tissue injury can affect biochemical values
- *Pregnancy*-alters some reference ranges
- *Menstrual cycle*-hormone measurements will vary through cycle

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Questions?

THANK YOU

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