

Use of leukocyte differential patterns to optimize diagnosis of bovine intramammary infections:

evaluation of partitioned vs. non-partitioned testing

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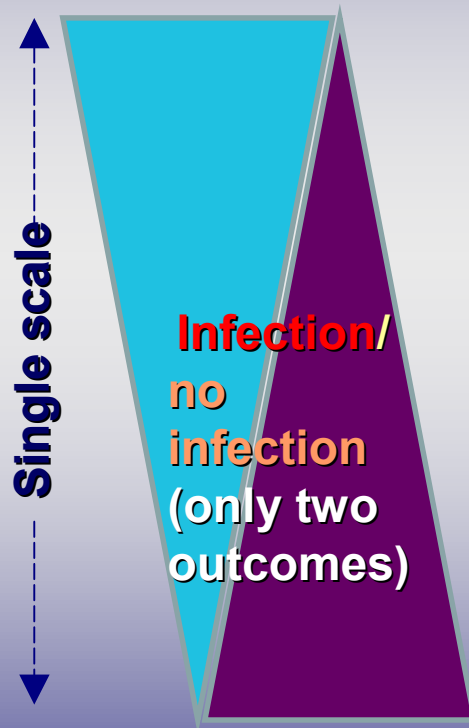
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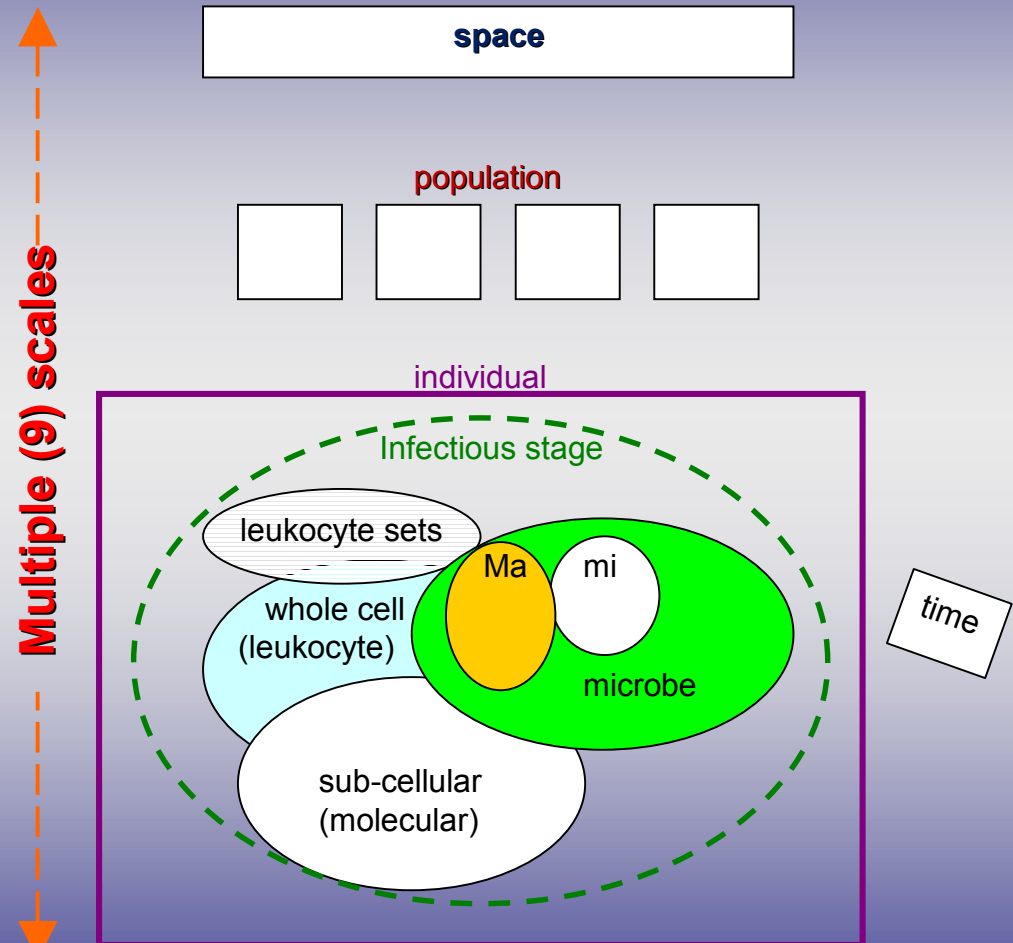
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One strategy to improve the diagnostic ability of a testing system is to **expand** the number of scales (variables) assessed

Classic paradigm
(binary, 2
outcomes)



New paradigm (multi-dimensional)



Supra-cellular leukocyte indices (e.g., phagocyte/lymphocyte percentages, Rivas et al, JVDI 13: 399-407, 2001)

EVALUATION

Purpose: to evaluate a diagnostic system for intra-mammary infections that

- a) considers *microbial*, *leukocyte* and *temporal* indicators
- b) does *not assume anything* (no particular *test* or indicator is assumed to be necessarily *correct* [no gold standard is assumed, although microbiologic testing is conducted], populations [e.g., herds] are not assumed to be identical, and no particular test *cut-off point* is assumed to be applicable to all populations), and
- c) is *adjusted to* the conditions of the *individual, subpopulation (disease stage), population, and time*.

Populations: 4 (“Studies 0-1-2-3”).

“Study 0”: a longitudinal, experimental study (n=6).

“Studies 1-3”: cross-sectional studies (2 US herds, 1 Israeli herd).

Materials & tests:

Each (mammary gland quarter) sample was investigated in terms of:

- a) whole leukocyte count (as estimated by the SCC),
- b) leukocyte differential counts, and
- c) microbiologic cultures (categorized as “no”, “minor” or “major” pathogen +)

Cross-sectional studies (all: n=1104)

Study I (n=120)

- * non-periparturient cows (regular milk)
- * 100 ul (duplicate) samples cultured
- * manual differential leukocyte counts (microscopy)

Study II (n=500)

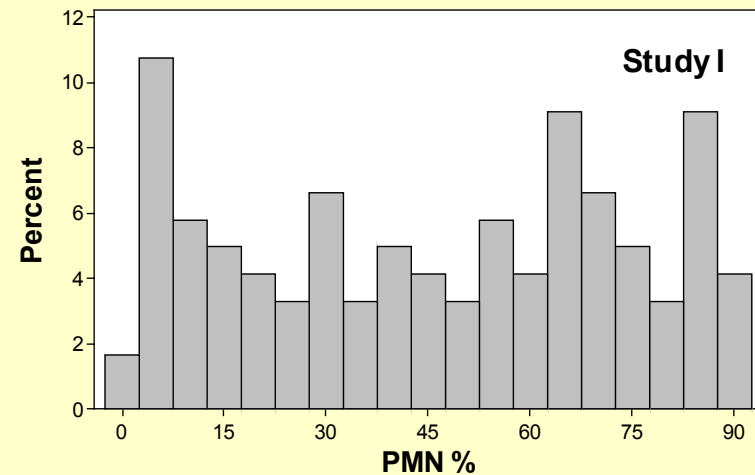
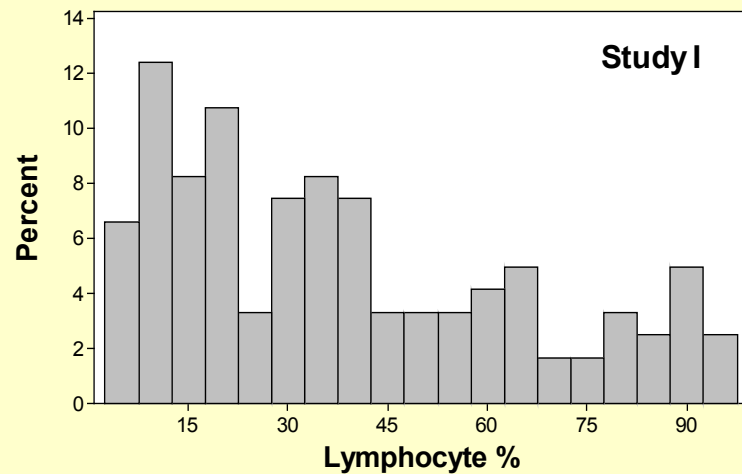
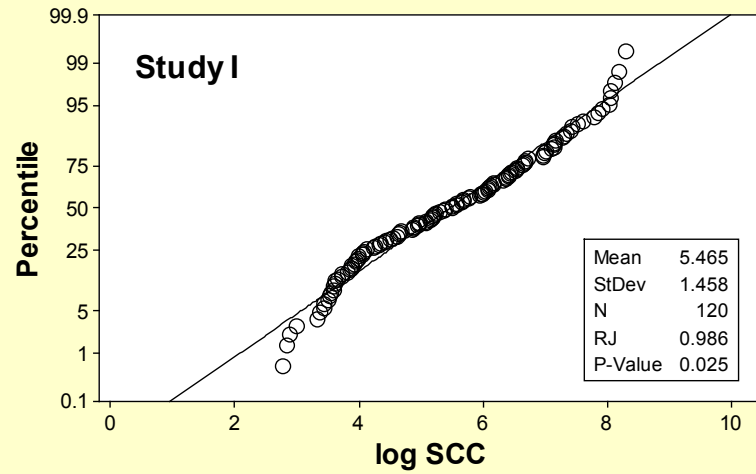
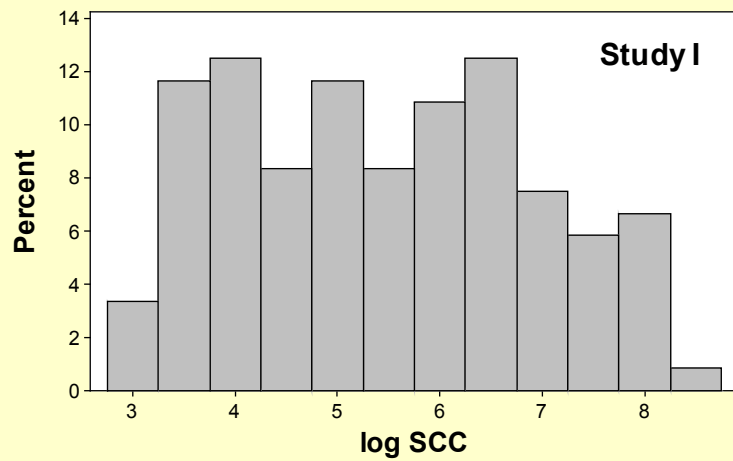
- * periparturient cows (colostrum)
- * 100 ul (duplicate) samples cultured
- * manual differential leukocyte counts (microscopy)

Study III (n=484)

- * non-periparturient cows (regular milk)
- * 10 ul (single) samples cultured
- * automated differential leukocyte counts (flow cytometry)

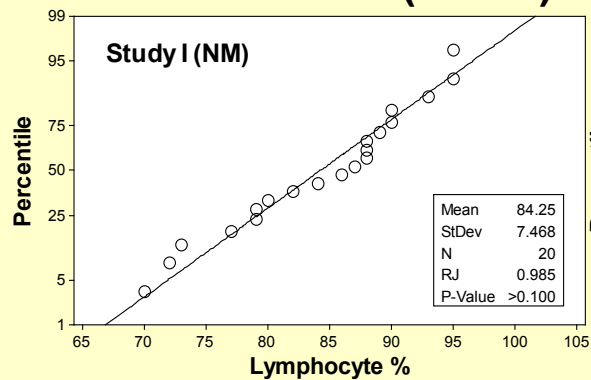
Results

Regardless of which indicator was considered, the distribution of each **dataset (herd)** was not linear.

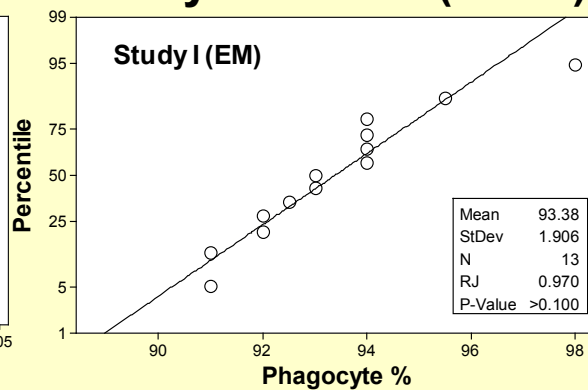


When each dataset (herd) was fragmented into **subsets** that (internally) showed similarities while (across) differed in at least 2 indicators, **6 subsets** were found in each of the 3 populations tested at a single time point. Each subset showed **linearity**. Tentative descriptors were assigned (one disease negative; and several disease positive [early and late disease]). Four “late” disease stages were observed.

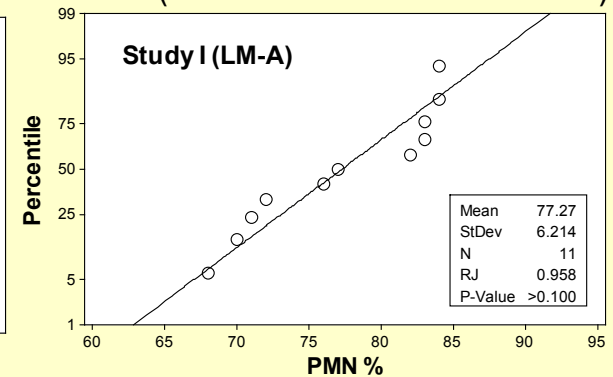
No infection (“NM”)



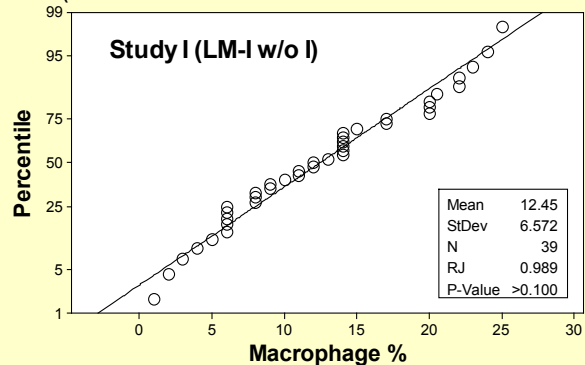
Early infection (“EM”)



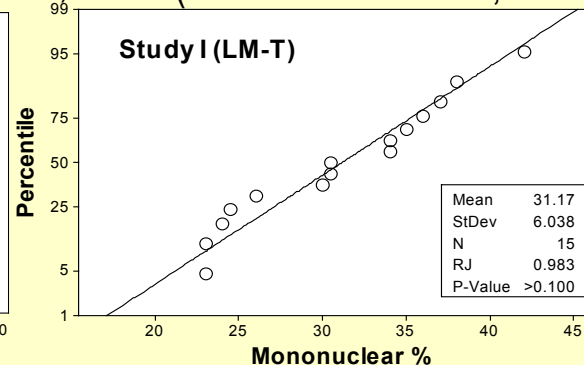
LM-1 (late active infection or LM-A)



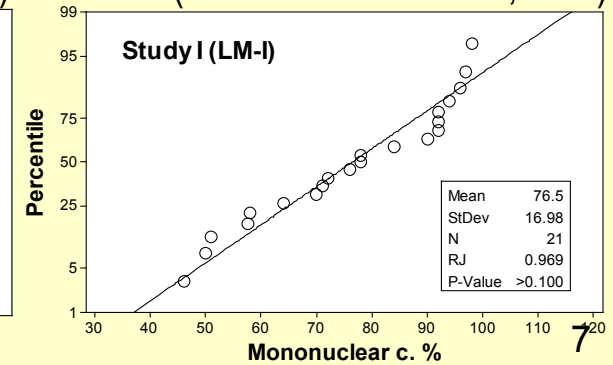
LM-2 (late infection without inflammation, LM-I w/o I)



LM-3 (late ‘transitional’ inf., LM-T)

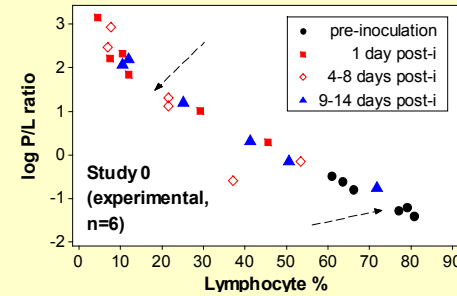
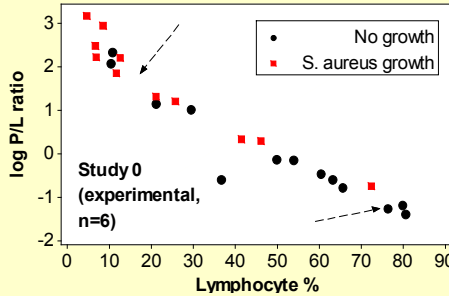
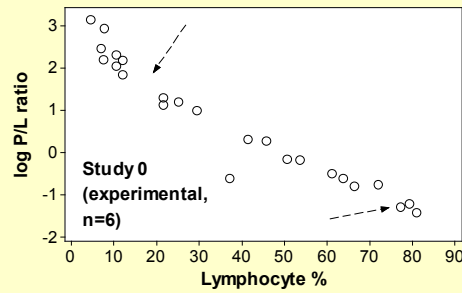


LM-4 (late inactive infection, LM-I)

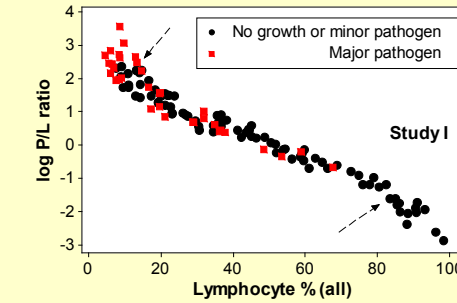
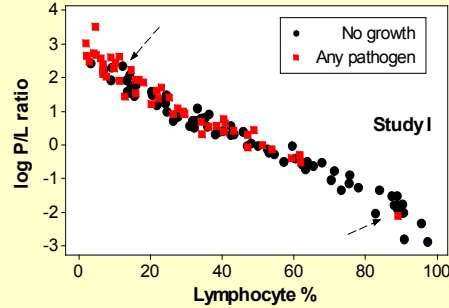
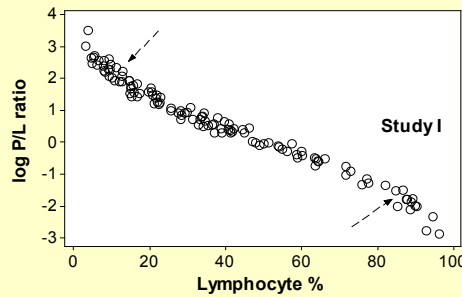


All 4 populations, which differed in prevalence, showed the same pattern (neither random nor linear): **major pathogens on one end, no infection on the opposite end.**

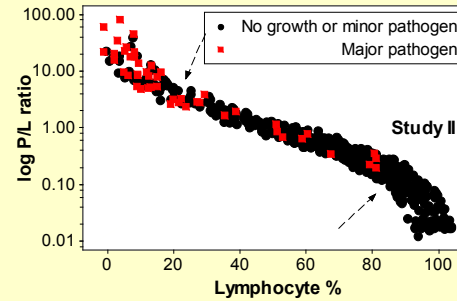
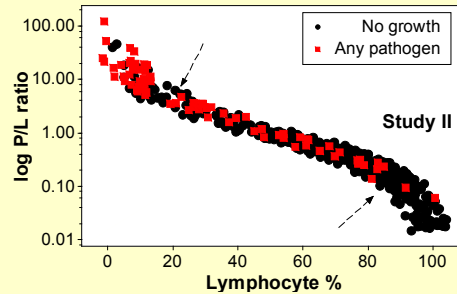
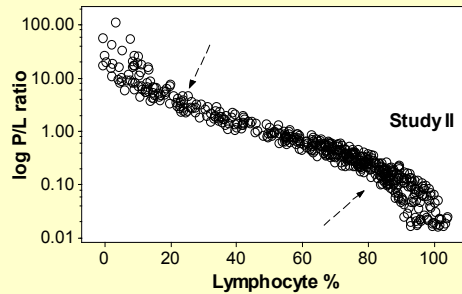
(Arrows indicate all populations showed 2 inflection points)



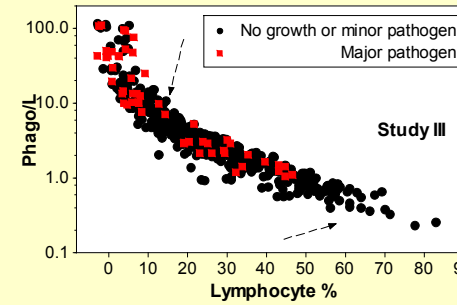
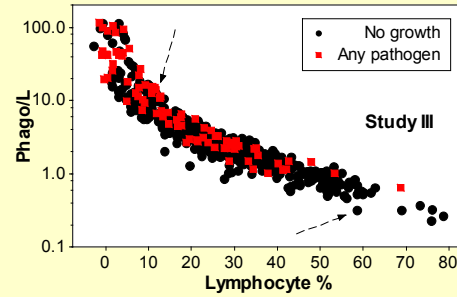
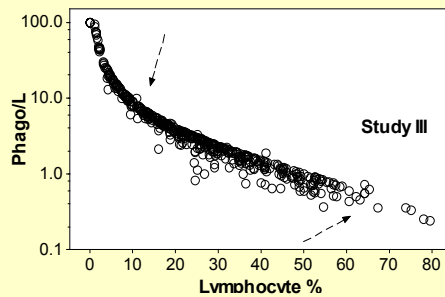
Preval.:
60 %



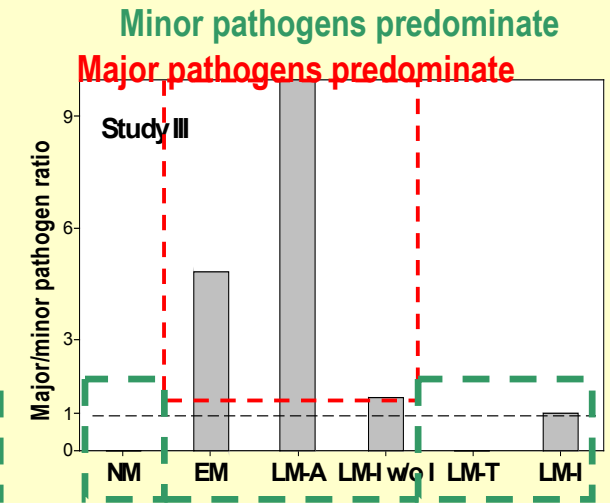
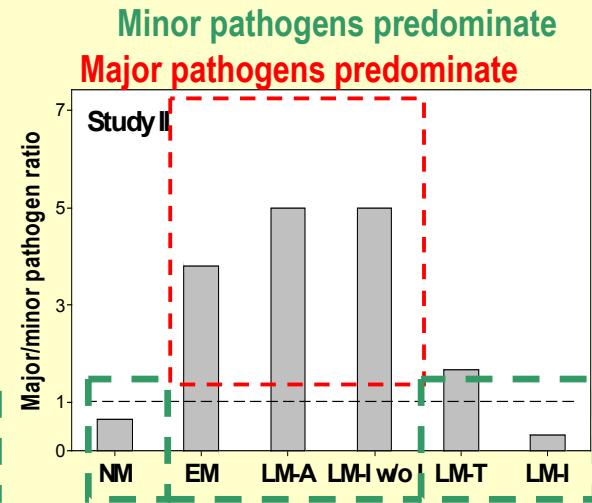
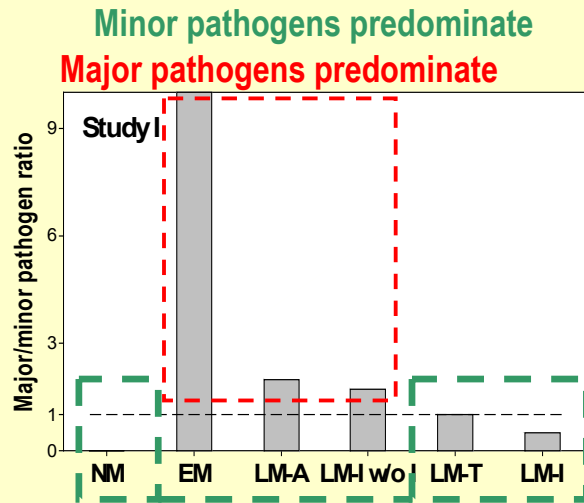
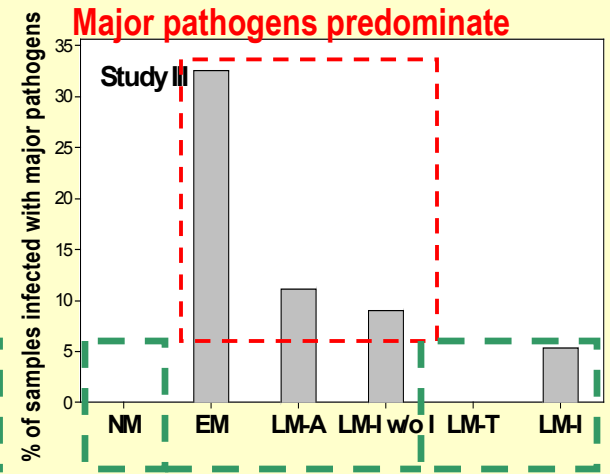
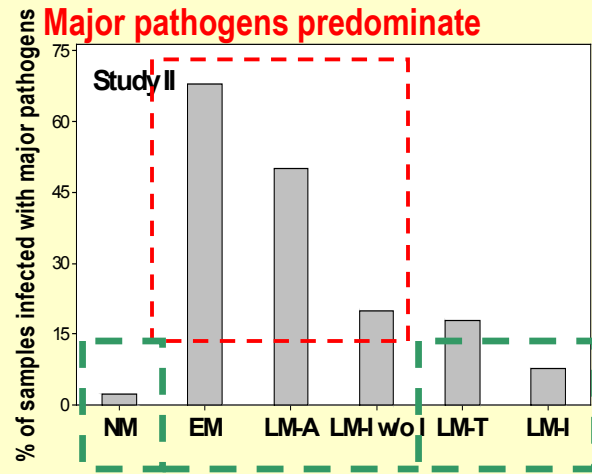
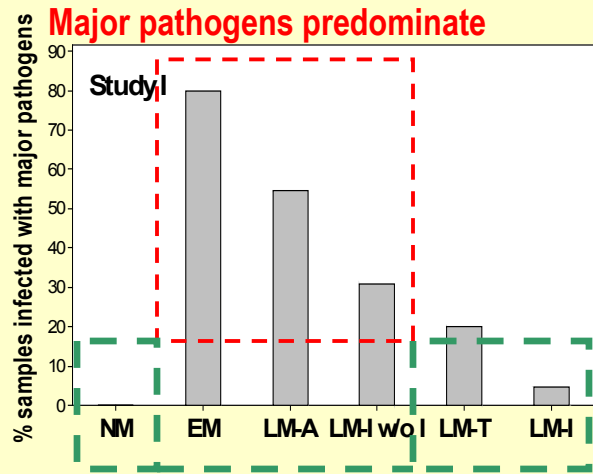
Preval.:
40 %



Preval.:
<20 %



Preval.:
<20 %



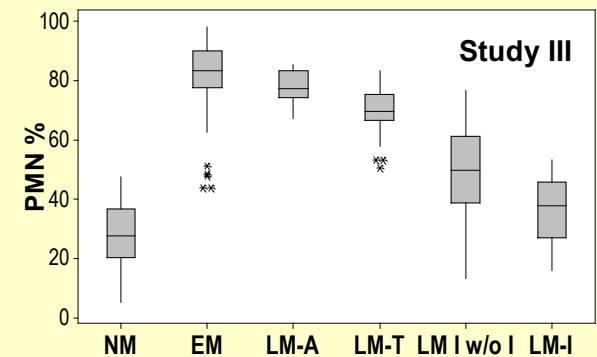
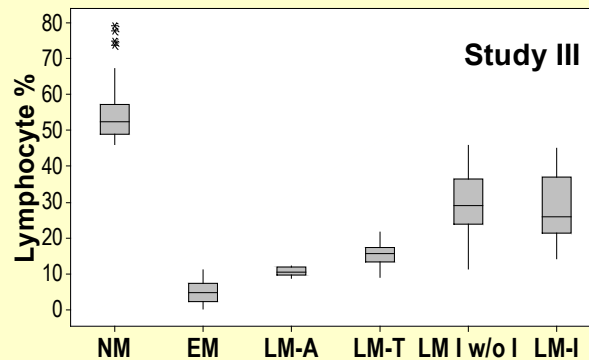
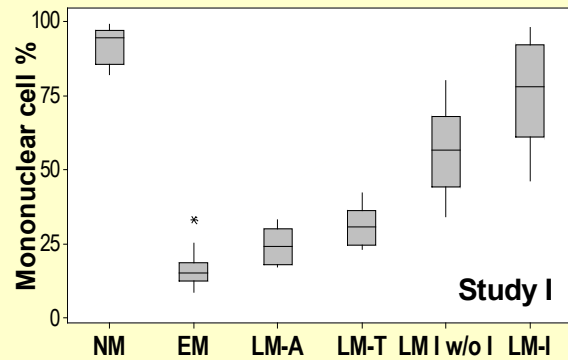
Minor pathogens predominate

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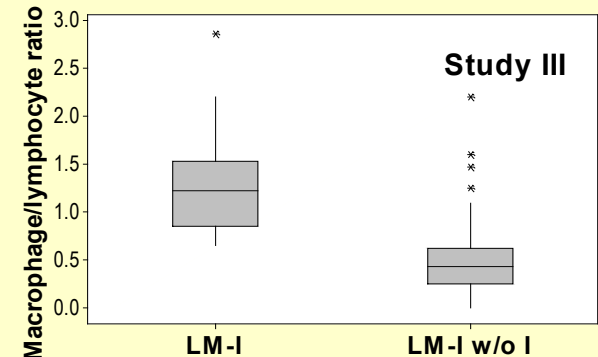
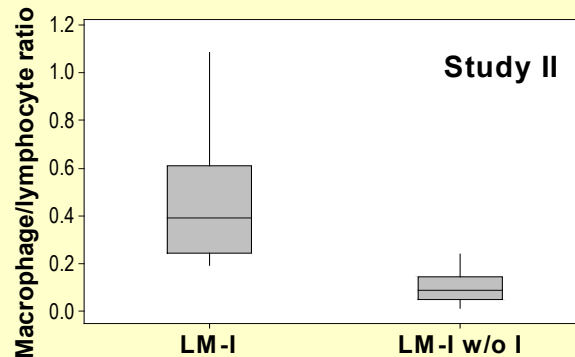
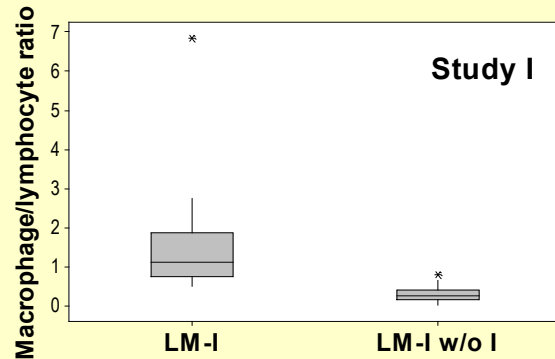
Minor pathogens predominate

Whether expressed as % of major pathogens, or as major/minor pathogen ratio, **microbial profiles differed across subpopulations (disease stages).**

Used collectively, leukocyte indicators differentiated all 6 disease stages



However, not all indicators applied to all populations.

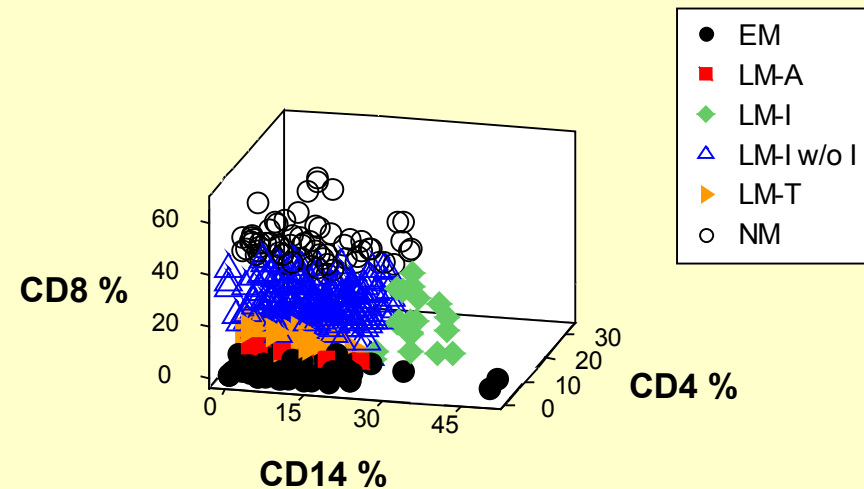


How can we estimate *time* with **cross-sectional** data?

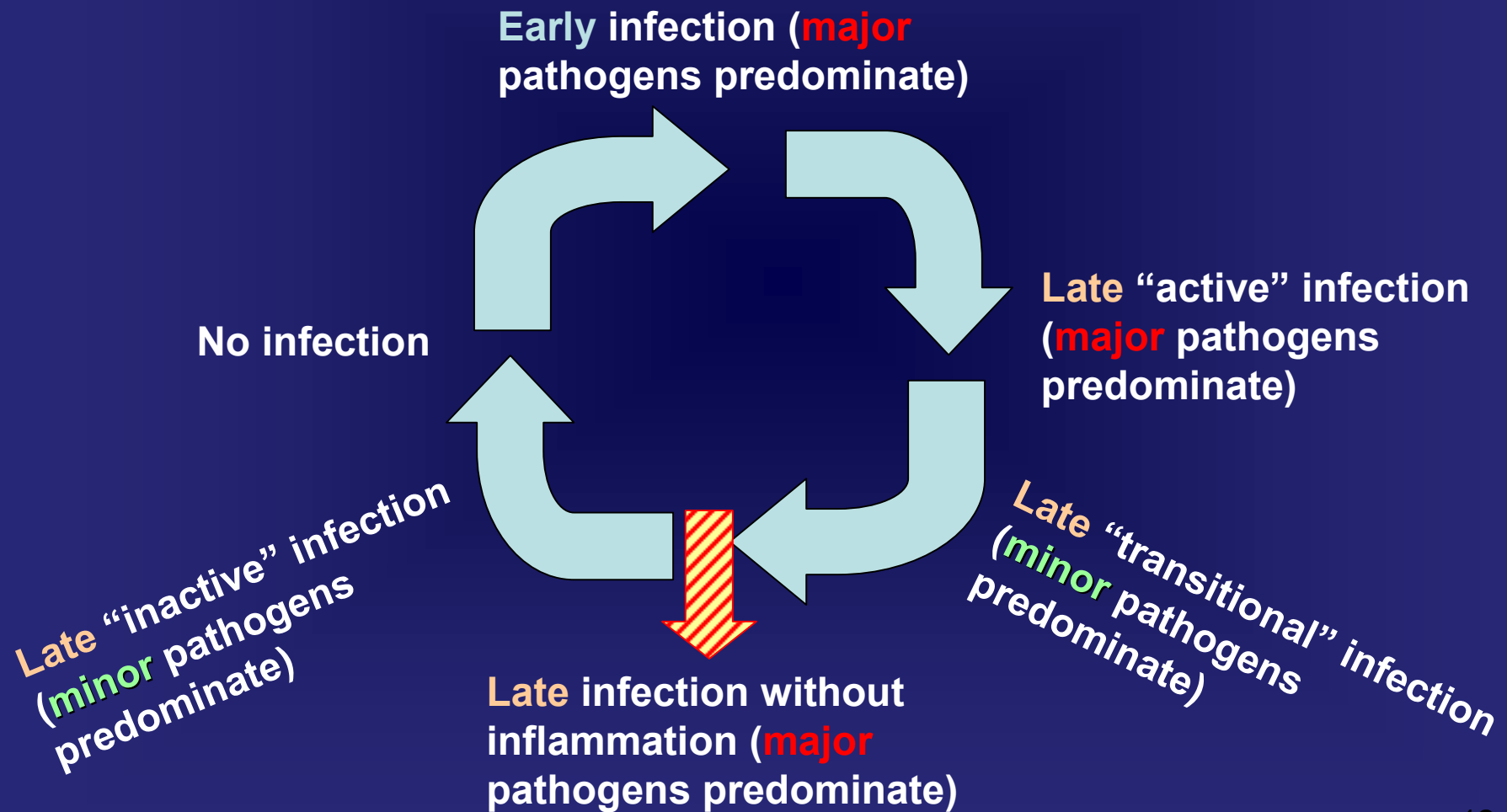
- CD8 predominates in healthy cows
- CD4 predominates in early mastitis
- CD8 predominates in late mastitis

(Rivas et al, CJVR 64:232-237, 2000).

Molecular data confirmed the temporal descriptors: all 6 disease stages showed non-overlapping ranges.



Are infections binary, linear or... (quasi) circular processes?



- How **accurate** is this system?

- Compared to the SCC and using microbiological results as reference, the total percentage of D– and D+ samples correctly diagnosed by the partitioning-based method was always **higher** than the SCC.

Test	Total accuracy (%)		
	Study I	Study II	Study III
SCC	75.8	84.0	98.1
Disease stage-based (micro-leukocyte profile-based) test	91.7	92.6	98.6

Summary

- Based on features shared by all populations (e.g., a sigmoidal distribution), which may **differ** across populations in specific values (e.g., the **location of inflection points**), we **fragment datasets** (population data) into **subsets**.
- Each subset (each disease stage) is characterized by (i) **continuous data** on [a] **bacterial** profile (e.g., major/minor pathogen ratio), [b] up to **15 leukocyte** indicators (e.g., the mononuclear/lymphocyte ratio); (ii) **relative time** (“inflammatory time”, e.g., “early”); and (iii) a **prognosis** (e.g., if “late ‘inactive’ infection” → recovery, e.g., it does not require treatment).
- Because it does not depend on any individual indicator, **this test can identify subsets previously missed by other tests**. E. g., the “late infection without inflammation” subset (usually, > 10% of all samples, with major pathogens but no inflammation) tends to be missed by the SCC.
- Using additional statistical tests, **each sample of each subset** is diagnosed by considering the **optimal cut-off** of the **optimal indicator** (whatever it may be). The indicator and cutoff used are selected **after data collection** (**procedures are adjusted to the tested population**).
- Results are **herd-specific, disease-stage specific, indicator-independent, cutoff-independent, prevalence-independent**.

Köszönöm!