

# The Natural History of Seminal Leukocytes in Men Seeking Infertility Evaluation

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# • Introduction

## Leukocytospermia

- True significance: still unknown
  - No detrimental effects
  - Impaired semen parameters (esp. sperm morphology and motility)
  - 1 ~ 3 million /mL (M /mL) → beneficial for sperm function (effects of cytokines / scavenging of abnormal sperm)
- Traditional WHO definition: > 1 Million /mL leukocytes

## Multifactorial origin

- Genital tract infections
- Smoking
- Alcohol consumption
- Marijuana use
- Prolonged abstinence
- Varicocele or vasovasostomy
- Certain sexual practices  
(use of vaginal products or anal intercourse)

→ ↑ WBC in semen  
→ Removal of defective sperm from the ejaculate (protective mechanism in abnormal spermatogenesis)

- Leukocytospermia

- ⇔ Presence of bacteria in the semen... ..*many studies*

- ⇔ (significantly) bacterial counts... .. *Punab et al*

- Threshold value for leukocyte count: 0.2 M /m l

- < Traditional WHO definition

- Any level of seminal leukocytes of 0.2 ~ 1 M /m l

- ⇔ Pathological ↑ of seminal reactive oxygen species

- low levels of seminal leukocytes

- ⇔ sperm DNA damage



## Decision to treat infertile patients

- Newly suggested **cut off** value for leukocytospermia → depends on identification of the **cause**, the **prognosis** and the **natural history** of leukocytospermia

♪ Natural history of leukocytospermia: One prospective study, traditional threshold of 1 M /m l

♪ This study: focused on monitoring the trend of seminal leukocytes levels over time in infertile patients with leukocytes  $\geq 0.2 \text{ M /m l}$

# • Materials and Methods

- Retrospectively review
- Cleveland Clinic Male Infertility Center
- Men presenting for a fertility evaluation
- September 2006 ~ January 2008
- Patients with no prior treatment

## Leukocytospermia

- Calculating the number of **round cells** /HPF
- **→**  $> 1 \sim 2$  /HPF **→ Endtz test**
  - Positive myeloperoxidase staining
  - Differentiate leukocytes from immature sperm cells
  - Leukocytospermia: Seminal WBC  $\geq 0.2$  million / ml
- **→** Subsequent assessment at a  $\geq 21$  days interval (Changes in the levels of seminal leukocytes)



## Analysis

- Differences in semen leukocyte levels
  - *Across all time periods* → Kruskal Wallis rank test
  - *Between individual time periods* → paired t-test
- Rate of spontaneous resolution
  - Resolution:  $\downarrow < 0.2$  million/ml
- Sequential  $\uparrow$  or  $\downarrow$  in leukocyte levels over time
- P value  $< 0.05$  -- statistically significant Statistical software, S-plus 7.0 (Insightful, WA)



# • Results

- 33x infertile patients: leukocytospermia
- 81x **Endtz tests** (2-5 /patient) prior to giving any Tx
  - → 48x **intervals** among patients
  - → compare ↑ or ↓ in semen leukocyte level
- Average time between Endtz tests: 151 days (21 - 1421)
- Average patient age: 35.2 years (23-48)
- 22/33 (67%): 1° infertility; 11/33 (33%): 2<sup>nd</sup> infertility
- 11x unilateral grade 1 or 2 left varicocele, and
- 1x bilateral varicoceles (R't grade 1 + L't grade



# • Consecutive Endtz tests

## analysis

Individual incremental changes

- Absolute leukocyte concentration: 23 ↑, 17 ↓, 8 —
- 7 / 33: semen leukocyte levels fall < 0.2 M / ml
  - Spontaneous resolution rate: 21 %
  - 3 / 7: leukocytospermia returned spontaneously



# • Discussion

## Current controversial outcome

- Impact of seminal leukocytes on male infertility and their etiology → still a subject of considerable debate
- **Rodin et al** no correlation leukocytospermia
  - ⇔ presence of **bacteria** on semen culture
  - ⇔ **semen parameters**
- **Lackner et al** leukocyte ⇔ seminal sperm parameters
  - $0 \sim 1 \text{ M/mL}$ : correlated positively
  - $> 1 \text{ M/mL}$ : deterioration

• Leukocytes: A response to bacterial infection / Scavenge abnormal germ cells

→ First by *Kiessling* in 1995

→ *Kaleli et al*: sperm from semen samples with leukocyte  $1 \sim 3 \text{ M/ml}$  → performed better on certain measures of sperm function (such as acrosome reaction and hypo-osmotic swelling test)

→ *Others*: Leukocytospermia → Inversely associated with motility and percentage of normal forms

leukocytospermia  $\Leftrightarrow$  actual bacterial infection of the genital ducts

- *Gdoura et al*, 116 patients (Seminal leukocyte  $\wedge$  bacteria)

- Establish the minimum leukocyte count

$\rightarrow$  significant bacteriospermia

- Cut off level of Leukocytes

$\Leftrightarrow$  sensitivity  $\wedge$  specificity for detecting

bacteria

$\rightarrow > 1 \text{ M /m l}$ : 20.3%  $\wedge$  81.5%

$\rightarrow \geq 0.275 \text{ M /m l}$ : highest ratio + A significant

correlation bacteriospermia  $\Leftrightarrow$  leukocytospermia

$\rightarrow$  A possible new cut-off level



leukocytospermia ↔ actual bacterial infection of the genital ducts

- Confounder: High level of skin contamination ∇ difficulty obtaining an accurate specimen
- Many studies: the presence of seminal leukocytes ↔ bacteriospermia
- Others: seminal leukocyte count significantly ↔ number of different microbes ↔ total microbial count
  - Using a receiver operating curve (ROC)
  - 1 M/ml (traditional threshold for leukocytospermia): low sensitivity

→ 2.5 M/ml (traditional threshold for bacteriospermia): low specificity

## Different threshold

- WHO threshold for leukocytospermia: 1 M /m l

→ Peroxidase positive cells

- Total number in the ejaculate → may reflect the severity of an inflammatory condition

- Cut-off values in fertile men 1 M ~ 2 M /m l

→ outcomes of semen quality / results of IVF

→ some consider very low; some consider very high

## ↑ oxidative stress / DNA damage

- low level of leukocytospermia ↔ no seminal leukocytes → ↑ oxidative stress in semen *Sharma et al*
- leukocytes + immature germ cells → major source of ROS in human semen *Other studies*
- Generation of ROS
  - Cellular mechanisms: Leukocytes = spermatozoa
  - In Leukocytes: **physiological necessity**, release large amounts of superoxide into phagocytic vesicles during the killing action of pathogens
- Sperm DNA damage ↔ leukocytospermia



what are the proper treatments for infertile men who have leukocytospermia ?

- First treat the cause ( Clinical assessment + proper microbiological work up → nature of leukocytospermia )
- Environmental culprits (smoking, alcohol, marijuana ) should be discontinued

- A study for Accessory gland infection (ex. prostatitis )

→ 102 men, leukocytospermia (Bryan-Leishman stain )

→ Abx Tx → 4 groups according to the treatment

1. with no treatment
2. antibiotic treatment alone
3. frequent ejaculation alone
4. antibiotic treatment with frequent ejaculation

→ Compared for resolution of leukocytospermia

- Significant resolution in all treatment groups at 1 month (↔ no treatment)
- Antibiotic treatment, frequent ejaculation, and antibiotic treatment + frequent ejaculation
  - All effectively treat leukocytospermia immediately after the treatment phase
  - Antibiotic treatment + frequent ejaculation → better resolution of infection (after 3 months of intervention)



- Patients with low level leukocytospermia → Indicated Empiric treatment with antibiotics and antioxidants
  - Disadvantages: emergence of resistant bacteria, toxic effects on sperm function
- **Lackner et al** 43% leukocytospermia resolved without treatment, WHO threshold of 1 M /m l (↔ threshold of 0.2 M /m l. 21% resolution)

- semen leukocyte levels (newly suggested cut off value) plotted over time → **no clear trend upwards or downwards** (low level of spontaneous variation)
- When defined by a lower threshold value
- Leukocytospermia seems to persist over time / Did't resolve spontaneously
- This information can now be used to assess treatment outcomes of low level leukocytospermia
- The selected treatment → reduces seminal leukocyte level  $< 0.2 \text{ M/ml}$  ( $> 21\%$ ) → considered to be effective

## Proper antibiotic regimens for genital tract

### infections

- → Improve semen parameters, appropriate ↓ in seminal WBC count and ROS production
- → spontaneous pregnancy rates (40%) in infertile men with prostatitis and prostatic vesiculitis
- → 3 months after therapy discontinuation → some sperm parameters (seminal WBC concentration and ROS generation) improved in patients with prostatitis (PR) and prostatic vesiculitis (PV) ↔ no improvement in patients with prostatic vesiculitis epididymitis
- → combined use of immune modulators and antioxidants → protect the sperm during maturation



## limitations to this study

1. small sample size → may not extrapolate to larger populations
2. study population: single institution & geographical region → may not represent all groups of patients
3. etiology of leukocytospermia may vary with incidences of various causative agents in different regions
4. 21% spontaneous resolution rate → 3/7 recurrent leukocytes prior to any intervention



the true resolution rate may be lower

(number of patients was too small to draw any

# • In conclusion

1. True threshold value for treating significant leukocytospermia remains to be established
2. If low level leukocytospermia proves to be detrimental → natural resolution rate needs to be defined
3. The spontaneous resolution rate of 21% that we observed serves as background data against which the efficacy of future treatments can be compared.
4. Larger prospective trials → characterize the role of lower levels of leukocytospermia on fertility

•THANK YOU FOR YOUR  
LISTENING