Overview of role of immunologic markers in HIV diagnosis

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Background: Immunologic Diagnosis of HIV infection in infancy

- The only specific immunologic marker for HIV infection is serological demonstration of HIV antibody
- But, in infants, presence of HIV antibody is indicative of HIV-exposed status and NOT of HIV-infection, because of passive transfer of maternal antibody (IgG) through the placenta, which can persist until 18 mo. of age
- The direct and indirect effects of HIV on the immune system can potentially be used as surrogates for diagnosis of HIV infection in infants
- Immunologic assays are more useful for monitoring disease progression than for diagnosis in infancy

Prerequisites for Immunologic Diagnosis of HIV infection in infancy

- Knowledge of HIV- infection status of pregnant women is critically important
- An immunologic marker or function in HIV-exposed infants should be able to distinguish them from
  - –HIV- exposed uninfected infants
  - –Non-HIV-exposed uninfected infants
- Thus, “normal” age-appropriate values for the uninfected population need to be established

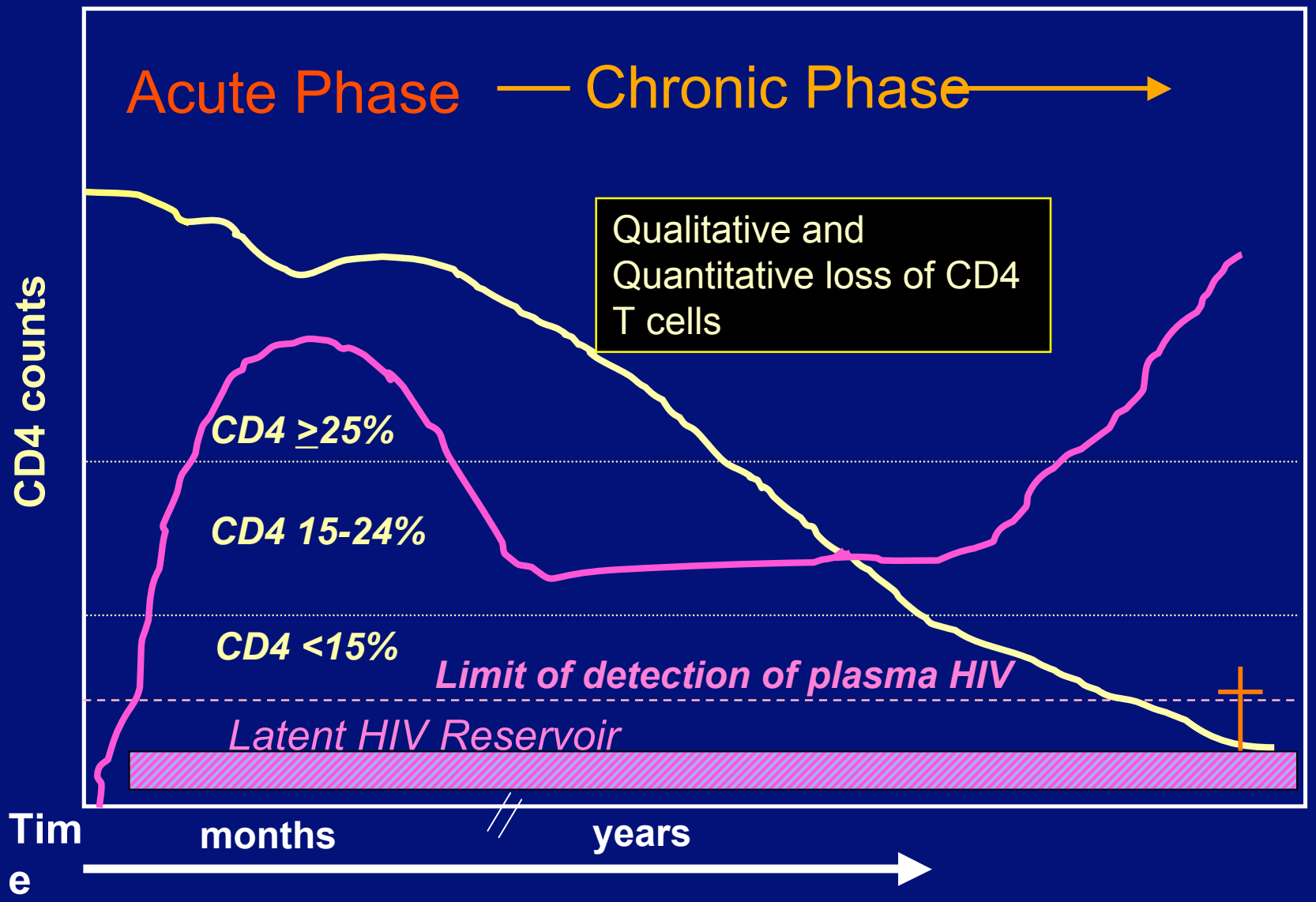
Potential Immunologic assays based on pathogenesis for diagnosis of HIV infection in infancy

- Assays that identify immune cell depletion
- Assays that identify HIV-associated cellular activation/immune dysregulation
- Assays that detect HIV-specific immune responses

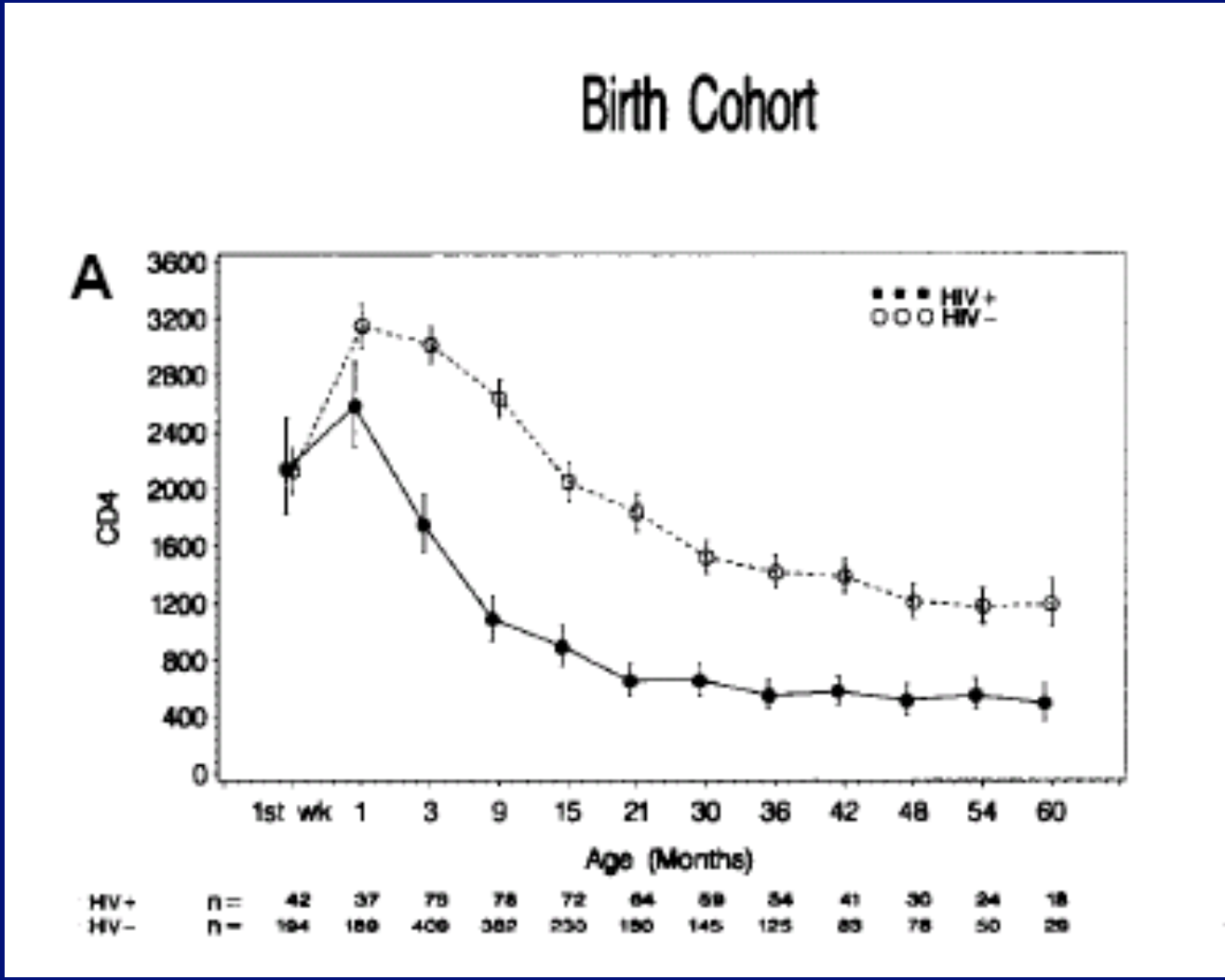
Potential compartments for immunologic assays for diagnosis of HIV infection in infancy

- Blood
  - Cells
  - Serum
- Secretions
  - Saliva ?
  - Urine ?
  - Gastric washings ?

CD4 T cell depletion in untreated HIV infection



Differences in CD4 counts in HIV-infected and uninfected infants can become apparent by 1 week

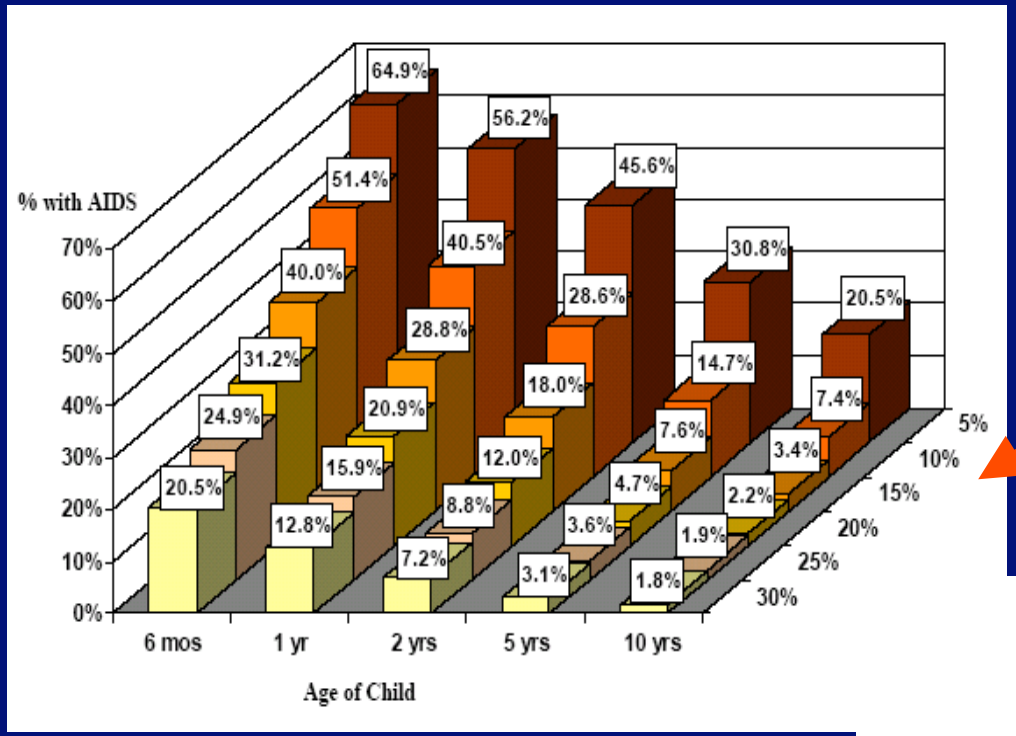


Prospective 5 year study of peripheral blood CD4+, CD8+, and CD19+/CD20+ lymphocytes and serum Igs in children born to HIV-1+ women, *Shearer et al, JACI, 106:559, 2000*

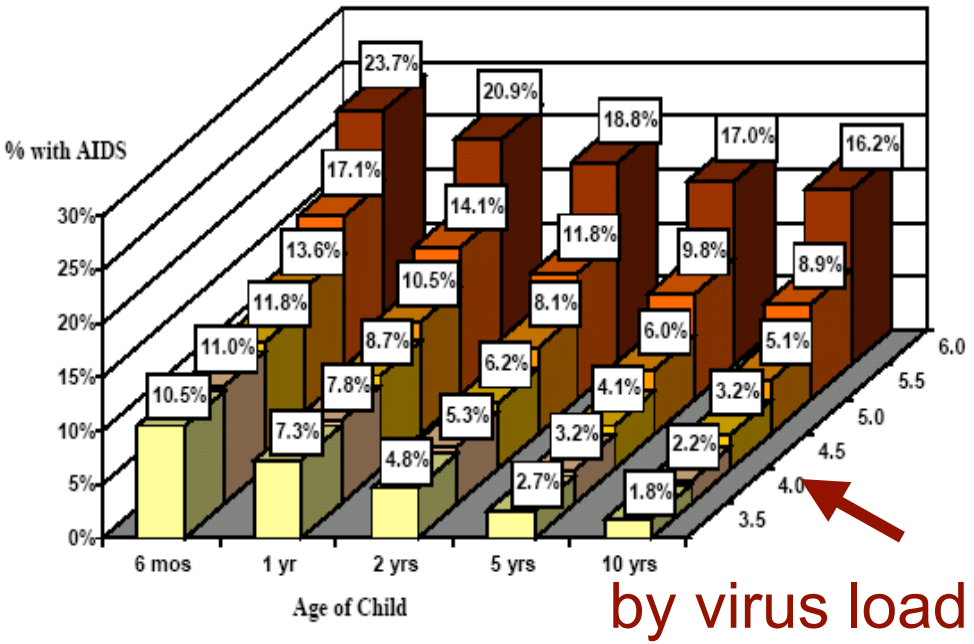
HIV and CD4 T cell depletion

- Studies in HIV-exposed infants have shown that CD4 T cells are decreased in HIV-infected infants in comparison to HIV-uninfected infants
- However, no clear CD4 cut-off's have been established for diagnosis of HIV infection, and debate about percentage and absolute values has not been resolved
- Normal values for CD4 counts may vary in different parts of the world
- CD4 has been invaluable as a tool for monitoring disease progression, and for guiding treatment initiation and response to therapy

Estimated probability of developing AIDS within 12 months at selected ages in HIV-infected children receiving no therapy or zidovudine monotherapy



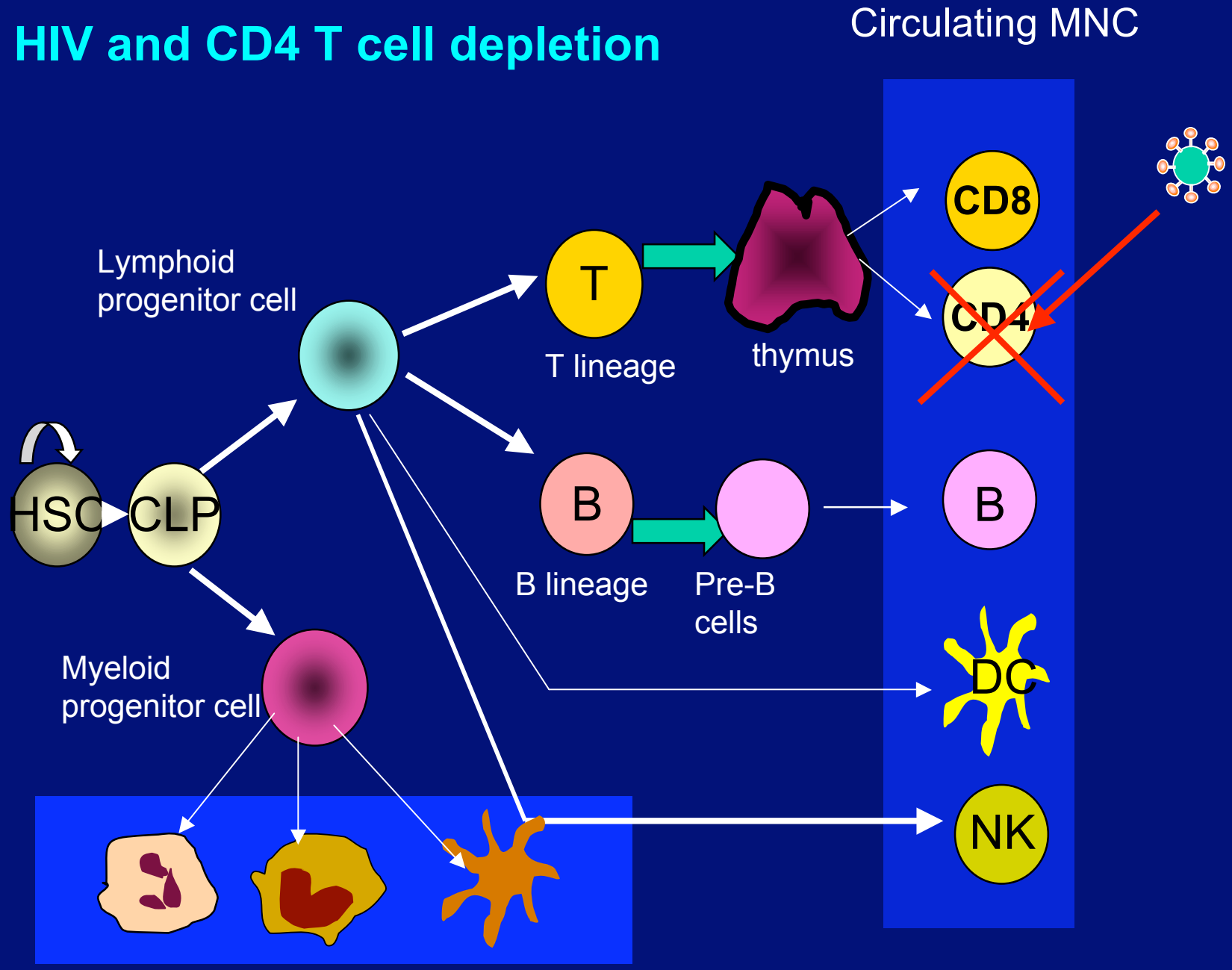
by CD4 %



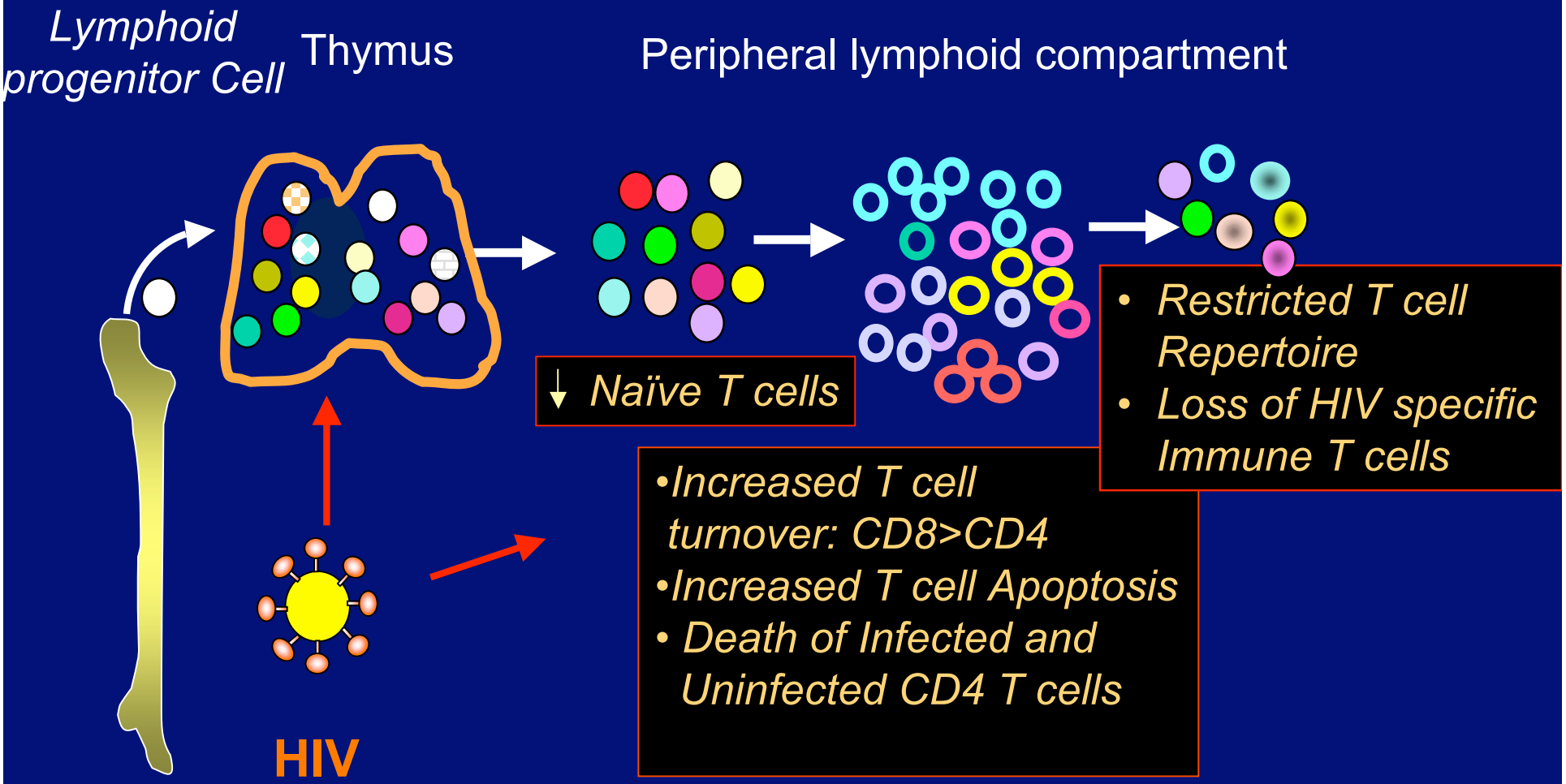
by virus load

From: Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection, March 2005
(Adapted From HPPMS publication in Lancet 2003: 362, 1605-11)

HIV and CD4 T cell depletion



Effects of HIV on T cells in the HIV infected host



Zimbabwe study: Zijenah et al (J Translational Medicine 3: 6, 2005)

Objective: Role of CD4:CD8 ratio in diagnosis

Subjects: Breastfeeding infants age <2 years, n=137 from 2 cohorts (AZT and PACD), Stratified 0-11mo and 12-18 mo.
2 mL blood one time sample collection

Labs:
Flow cytometry (Coulter Epics XL) and WBC and lymphocyte count (Celldyn hematologic analyser): %CD4, %CD8, absolute CD4 and CD8, CD4/CD8 ratio

DNA PCR (Roche v 1.5):
76+ (median age: 5.5mo.) and 61 negative (median age 8 mo.)

Zimbabwe study, Results

PCR+ versus PCR- infants:

- CD4 lower
- CD8 higher
- CD4/CD8 ratio lower

T cell subset profile of HIV-1 infected and uninfected infants

	PCR Positive (n = 76)	PCR Negative (n = 61)	*P* value[a]
Median age (months)	5.5 (IQR: 3–13)	8 (IQR: 4–14)	0.08
Median $CD4^+$(cells/μL)	521.5 (IQR: 323–805)	1356 (IQR: 916–1769)	<0.001
Median $CD8^+$(cells/μL)	1302.5 (IQR: 829–2054)	799 (IQR: 471–1020)	<0.001
Median CD4/CD8 ratio	0.4 (IQR: 0.3–0.6)	1.8 (IQR: 1.4–2.3)	<0.001
Median %CD4	13.9 (IQR: 9.2–19.1)	29.9 (IQR: 25.3–34.5)	<0.001
Median %CD8	31.3 (IQR: 22.3–42.9)	18.4 (IQR: 14.2–21.5)	<0.001

Abbreviations: PCR, polymerase chain reaction; n, number tested; *P* value[a] for statistical significance between group medians was estimated using the Kruskal-Wallis test.

Zijenah et al (J Translational Medicine 3: 6, 2005)

Evaluated parameters for CD4/CD8 ratio for the three infant age groups using DNA PCR as reference standard

	0–18 months infant age group (n = 137)	0–11 months infant age group (n = 95)	12–18 months infant age group (n = 42)
%Sensitivity	98.7 (CI: 96.1–100)	98.2 (CI: 94.7–100)	100 (CI: 100–100)
%Specificity	98.4 (CI: 95.2–100)	97.5 (CI: 92.7–100)	100 (CI: 100–100)
%PPV	96.4	94.6	100
%NPV	99.4	99.2	100
%TE	98.5 (CI: 96.5–100)	97.9 (CI: 95.0–100)	100 (CI: 100–100)

Abbreviations: n, number tested; NPV, negative predictive value; PPV, positive predictive value; TE, test efficiency.

Zijenah et al (J Translational Medicine 3: 6, 2005)

Association of Selected Phenotypic Markers of Lymphocyte Activation and Differentiation with Perinatal HIV Transmission and Infant Infection

Lambert JS et al Clin and Diagnostic Lab Immunol 12: 622, 2005

PACTG 185 cohort women (n=215); Infants (n=192)

Non transmitting Women
Higher CD3 CD4, lower CD8 CD38

Uninfected infants
Higher CD3 CD4, Naïve CD4

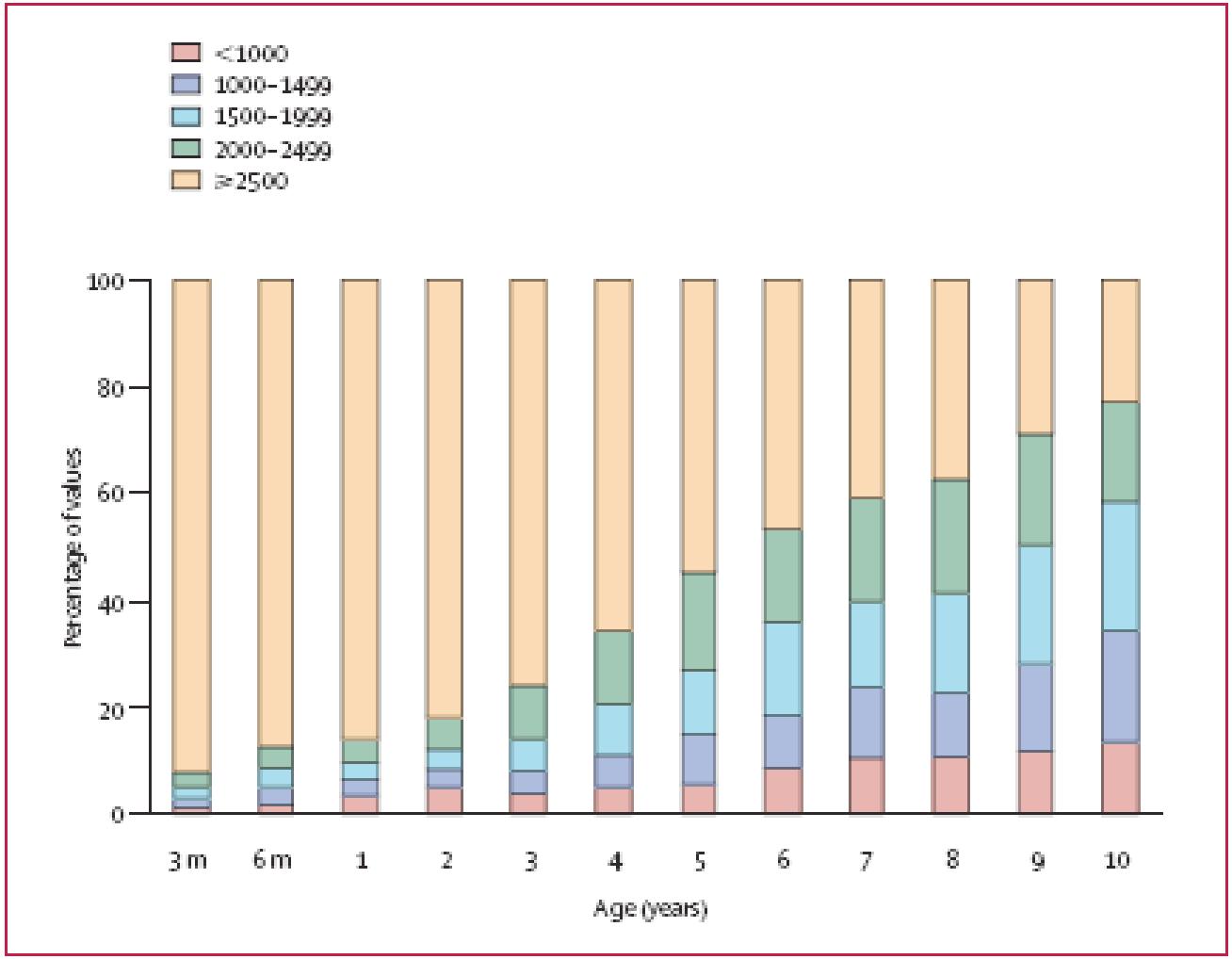
Infected Infants
Higher Total CD8, CD8 HLA DR, CD45RA HLADR and CD28 HLADR

Can we simplify immunologic testing?

What is the role of

- Total Lymphocyte Counts
- Serum assays
- Other compartments

Distribution of total lymphocyte count by age



Use of Lymphocyte Count for Informing when to start antiretroviral therapy in HIV-infected children: a metaanalysis of longitudinal data:

HPPMCS: Lancet, 366:1868,2005

Total Lymphocyte Count and HIV Disease Progression

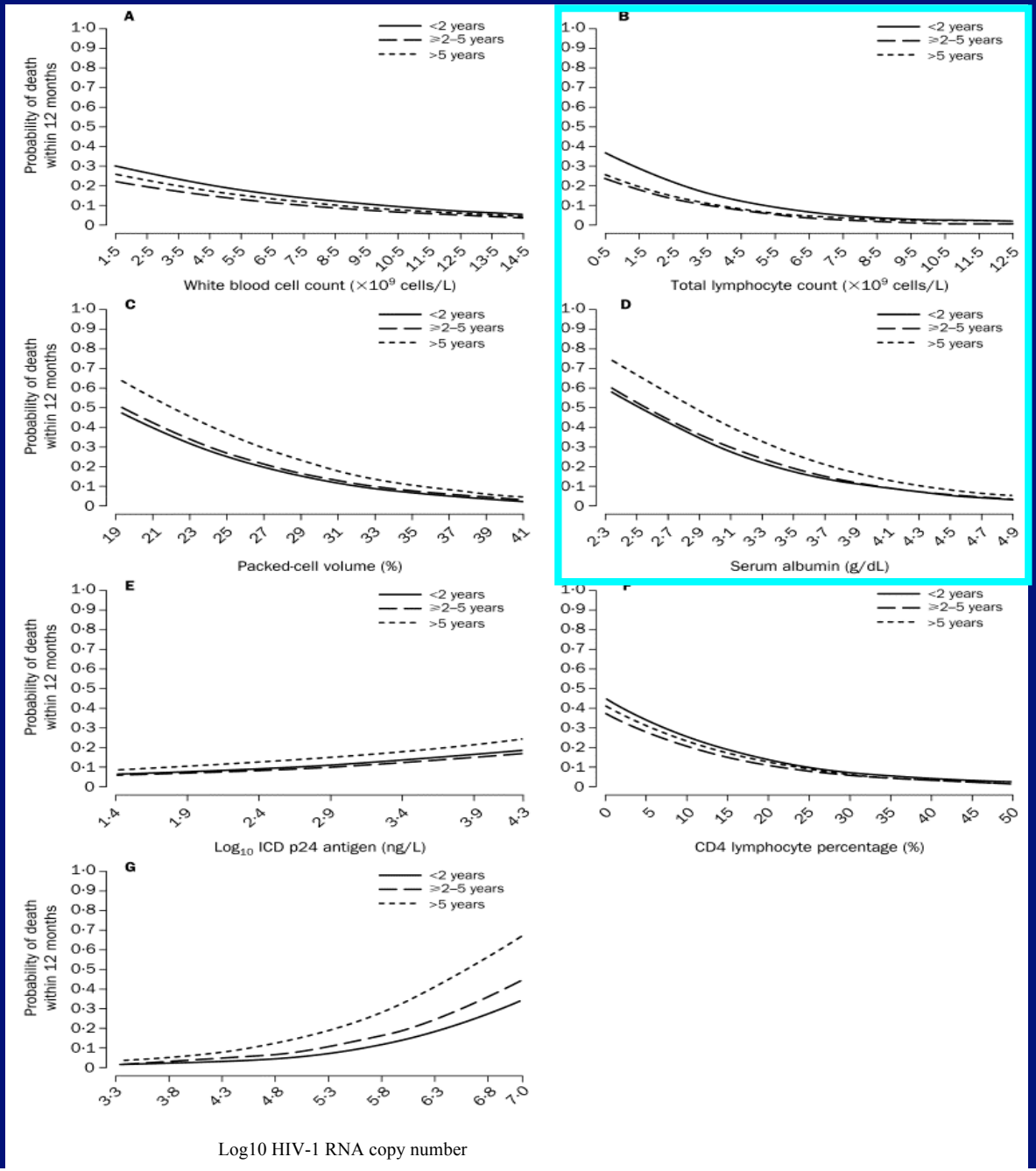
Age (years)	Endpoint	Estimated risk (%) at total lymphocyte count (95% CI)							
		500 per μL	1000 per μL	1500 per μL	2000 per μL	2500 per μL	3000 per μL	4000 per μL	6000 per μL
0·5	AIDS	69 (61–77)*	61 (54–68)	53 (48–59)	46 (41–50)	39 (35–43)	34 (30–38)	26 (23–29)	17 (15–20)
	Death	56 (46–68)*	44 (38–52)	34 (30–39)	26 (23–30)	20 (17–23)	15 (13–18)	9·4 (7·3–12)	4·6 (3·8–5·7)
1·0	AIDS	67 (58–73)*	55 (49–61)	44 (39–48)	35 (32–39)	28 (25–32)	23 (20–26)	16 (15–19)	11 (10–13)
	Death	52 (45–60)*	38 (33–42)	26 (24–29)	18 (16–20)	12 (10–15)	8·7 (7·0–11)	4·8 (4·0–5·8)	2·6 (2·1–3·1)
1·5	AIDS	64 (56–71)*	50 (44–55)	37 (33–41)	28 (25–51)	21 (19–24)	17 (15–19)	12 (11–13)	8·6 (7·7–14)
	Death	49 (44–55)*	32 (29–36)	20 (18–23)	13 (11–15)	8·2 (6·8–9·6)	5·5 (4·5–6·5)	3·0 (2·5–3·4)	1·9 (1·5–2·3)
2·0	AIDS	62 (53–68)*	45 (39–49)	32 (28–35)	22 (20–25)	16 (15–19)	13 (11–14)	9·0 (8·2–12)	7·3 (6·5–12)
	Death	46 (42–52)*	28 (25–31)	16 (14–18)	9·4 (8·1–11)	5·7 (4·8–6·6)	3·7 (3·1–4·3)	2·1 (1·8–2·4)	1·6 (1·2–1·9)
5·0	AIDS	50 (42–57)*	26 (22–29)	13 (12–15)	8·2 (7·1–9·3)	6·0 (5·2–7·0)	5·2 (4·5–6·2)	4·8 (4·0–5·7)	4·7 (3·9–5·7)*
	Death	34 (30–39)*	14 (12–16)	5·3 (4·3–6·4)	2·4 (1·9–3·0)	1·4 (1·1–1·7)	1·1 (0·8–1·3)	0·9 (0·6–1·2)	0·9 (0·6–1·2)*
10	AIDS	36 (29–43)	12 (8·5–15)	5·2 (4·2–6·3)	3·8 (3·1–4·6)	3·4 (2·7–4·3)	3·4 (2·6–4·3)	3·3 (2·5–4·3)*	3·3 (2·5–4·3)*
	Death	22 (18–26)	5·2 (3·3–7·2)	1·5 (1·0–2·3)	0·8 (0·6–1·1)	0·6 (0·4–1·0)	0·6 (0·4–0·9)	0·6 (0·4–0·9)*	0·6 (0·4–0·9)*

*Estimates are unreliable because these total lymphocyte counts are rarely recorded at these ages.

Table: Estimated risk of AIDS and death within 12 months at selected values of age and total lymphocyte count

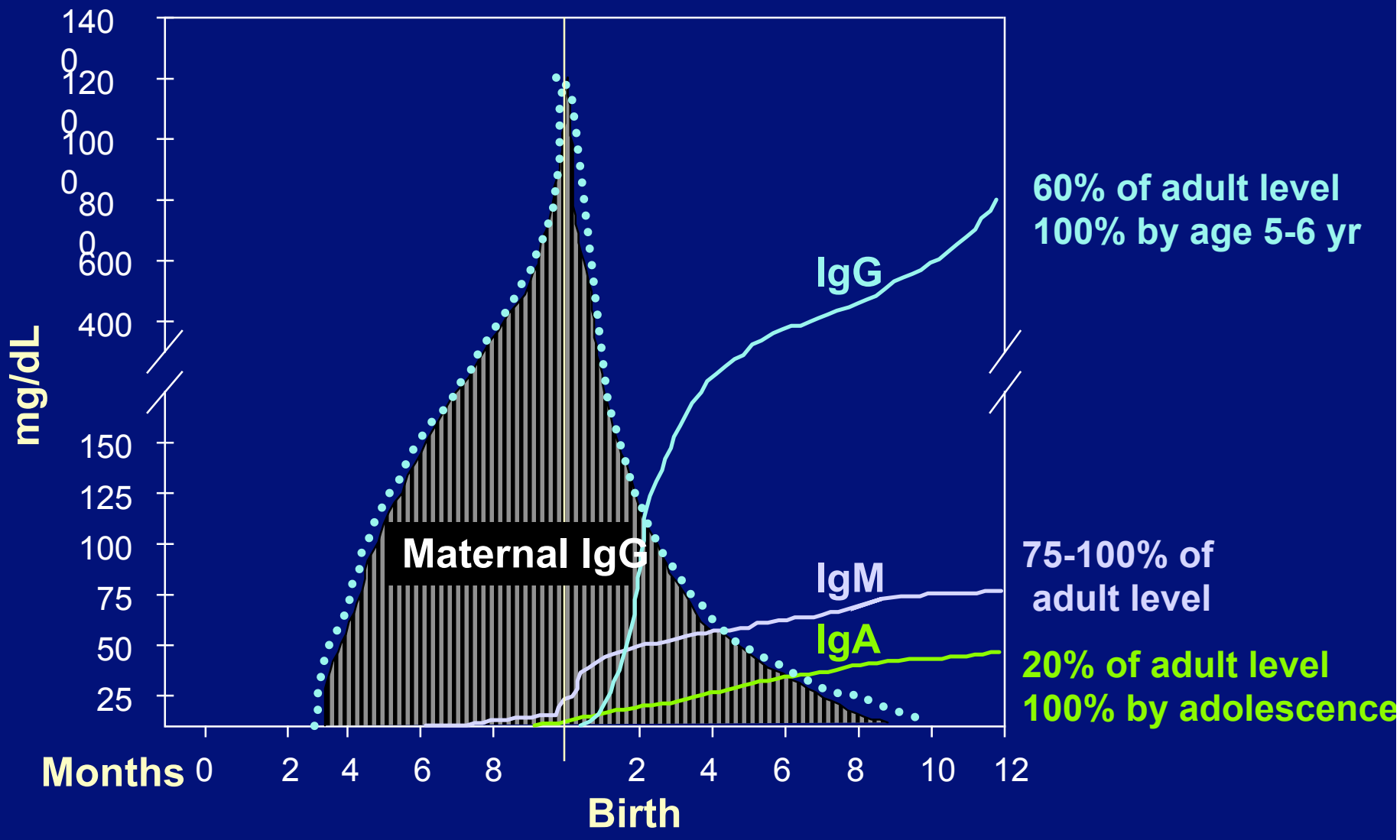
Estimated probability of death within 12 months per assay

"Alternatives to HIV-1 RNA concentration and CD4 count to predict mortality in HIV-1 infected children in resource poor settings":
Mofenson LM et al, Lancet 362:1625, 2003

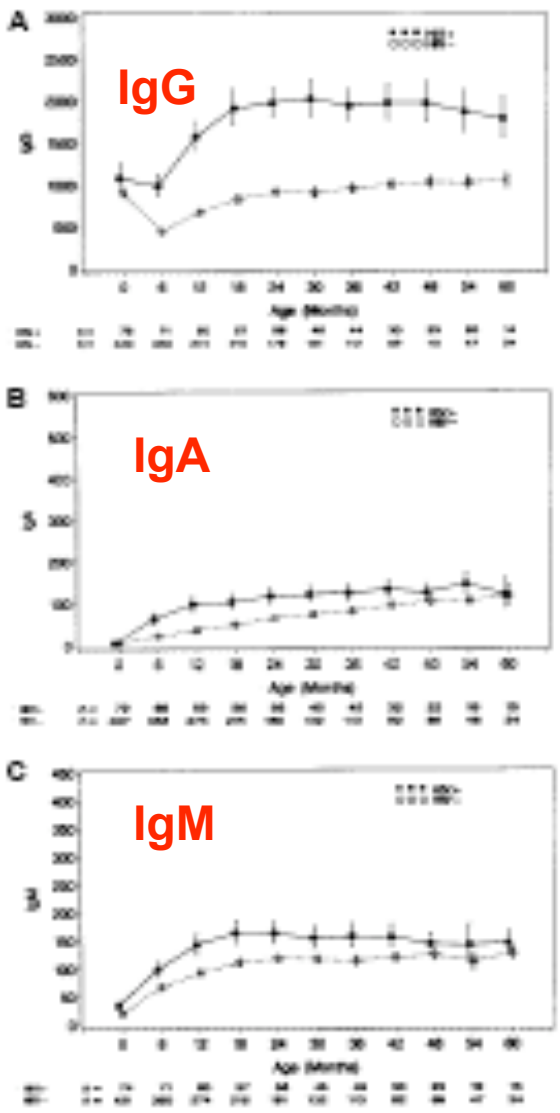


Serum Immunoglobulins

Immunoglobulin levels in the fetus and infant in the first year of life



Serum Igs in birth cohort



Prospective 5 year study of peripheral blood CD4+, CD8+, and CD19+/CD20+ lymphocytes and serum Igs in children born to HIV-1+ women

Shearer et al, JACI, 108:559, 2000

Results

Immunoglobulin levels were determined at 6 mo. Intervals from birth to 60 mo.

Ig levels in HIV infected infants were significantly higher than uninfected infants for

- IgG at birth to 60 months
- IgA after birth (6 mo) to 54 mo.
- IgM at birth to 42 months

Desai et al: CROI 2005
Sensitivity & Specificity of HIV IgA ELISA and p24 antigen ELISA in children < 18 months

	HIV DNA PCR POSITIVE	HIV DNA PCR NEGATIVE
HIV IgA +VE	30 (71%)	0
P24 Antigen	16(42%)	0
TOTAL n=100	42	58

In children <18 months, the sensitivity of HIV IgA v/s p24 antigen ELISA was 71% v/s 42% and specificity of both assay was 100 %

Summary 1
Potential immunologic diagnostic assays based on alterations in peripheral blood cellular composition

Reduction in

CD4 T cells;
Naïve CD4 T cells
CD4/CD8 ratio*

Increases in

CD8 T cells
Activated CD8 T cells

? Changes in

Total lymphocytes
Total T cells
Other cells
(DC/NK/B/?)

Summary 2
Potential Immunologic assays for diagnosis of HIV infection in infancy

Serum based

- Increased Immunoglobulins: Role of IgM/IgA?
- HIV antibodies: IgA?
- Other soluble markers: sFasL, $\beta_2$microglobulin neopterin, albumin etc

Other compartments?

- Saliva
- Urine
- Gastric washings.

  Role of rapid testing

Can we avoid drawing blood by venepuncture for immunologic studies?

Potentially Yes

- Heel stick in babies
- Finger stick in older children

Pediatrics. 1985 Dec;76(6):914 -7.

T lymphocyte subpopulations in high -risk infants: influence of age and blood transfusions .

Pahwa S, Sia C, Harper R, Pahwa R.

Methodology was established to analyze T lymphocytes and T cell subsets with monoclonal antibodies on microsamples of blood obtained by heel puncture in infants . Results in 39 high -risk infants indicated that during the early neonatal period they had a higher percentage of T4 -bearing cells and a lower percentage of T8 antigen - positive cells as compared with adults. These values progressively approached adult values, and at 3 to 7 months the T cell subsets and the T4/T8 ratios were within the adult range **. A significant inverse relationship was noted between the number of blood transfusions given to the infants between 2 and 12 weeks of age and the corresponding T4/T8 ratio** . These findings suggest that interpretation of T cell subsets in frequently transfused infants should take into consideration not only the age of the infant but also the poss ible influence of transfusions per se on the distribution of T cell subsets in the peripheral blood.

Tiered Immunologic diagnostic approaches for HIV exposed infants in the absence of specific diagnosis

- Cell surface marker analysis (based on resources):

3 colors: CD3/CD4/CD8
CD3/CD4/CD45RA
CD8/HLA-DR/CD38

2 colors: CD4/CD8
CD4/CD45RA
CD8/CD38

1 color: CD4, CD8, CD3

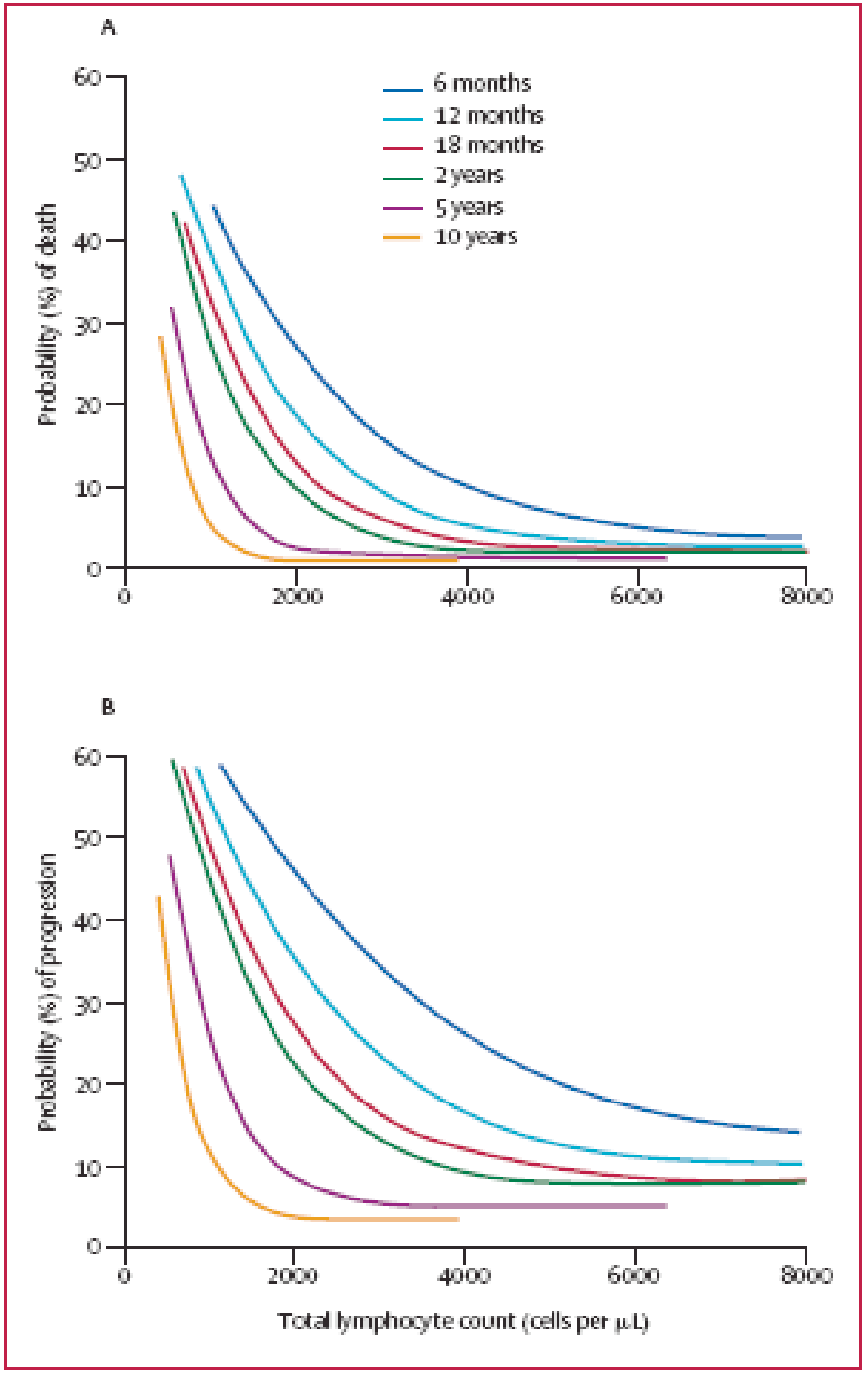
Loss of CD4 cells
Loss of naïve CD4 cells
Increase in activated CD8 cells
Assess CD4/CD8 ratio in all

- WBC counts: Lymphocytes
- Immunoglobulin levels: IgA, IgM, IgG in serum
- HIV antibody testing in serum and other compartments;
- Rapid testing
- Other Serum markers: sFas, neopterin, $\beta_2$microglobulin

Estimated risk within 12 months of:

A: Death

B: Progression to AIDS



Immunologic assays for diagnosis/monitoring of HIV infection in infancy

- Assays that identify cell depletion, with decreases of
  - CD4 T cells; Naïve CD4 T cells
  - CD4/CD8 ratio
  - Total T cells
  - Total lymphocytes
  - *Other cells (DC/NK/B/?)*
- Assays that identify HIV-associated cellular activation/immune dysregulation
  - Immune activation markers on T cells
  - Immunoglobulin levels
  - *Serum markers*
- Assays that detect HIV specific immune responses
  - HIV specific CD4/CD8 T cell responses