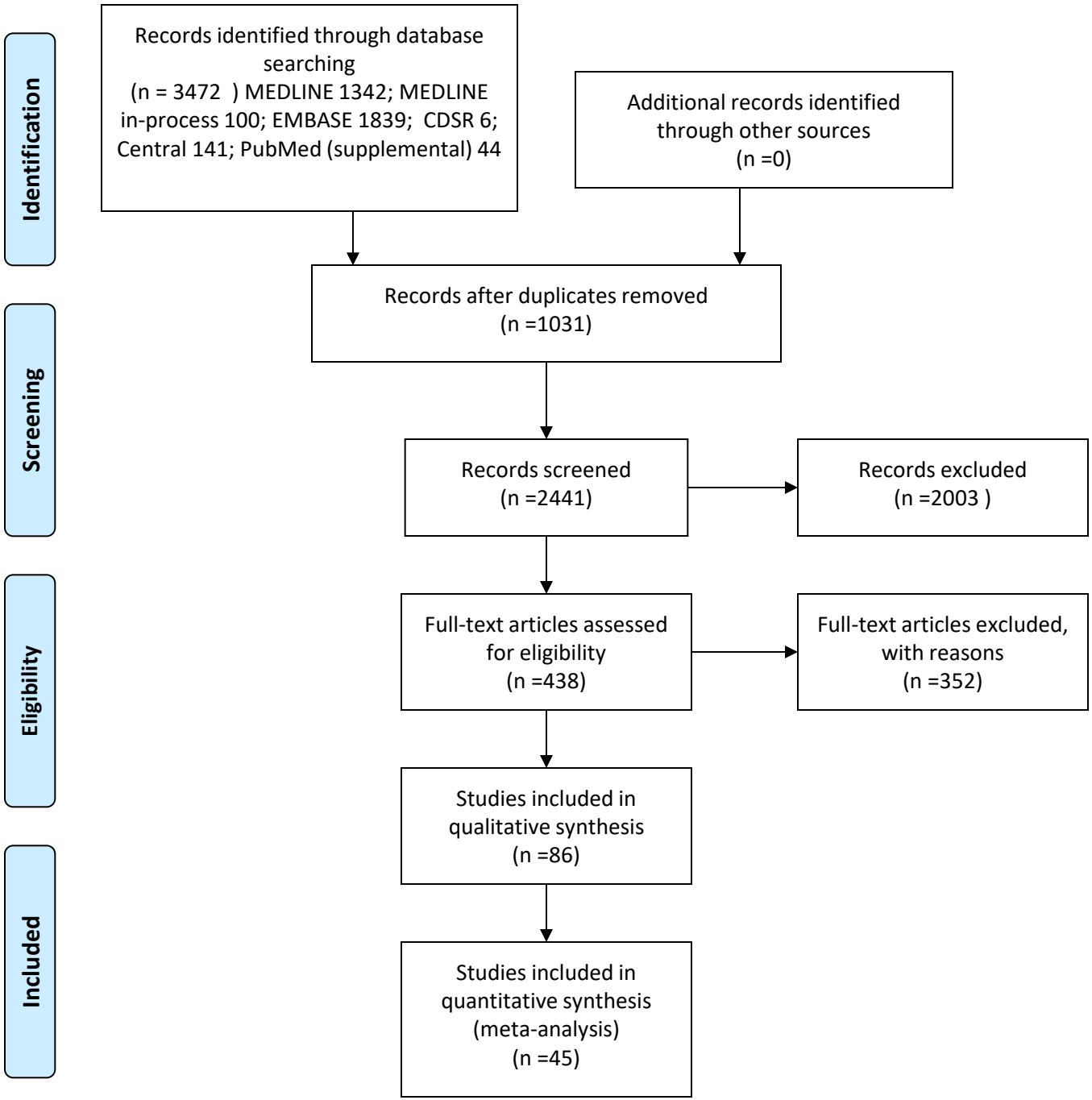
Systematic review in CMV viral load testing with PCR method in solid organ transplant patients

Yoichiro Natori/Atul Humar
Transplant Infectious Diseases
University Health Network

Preamble

- A systematic review of studies analyzing CMV viral load data in solid organ transplant recipients was undertaken.
- The search strategy is shown in the appendix.
- In order to contextualize the findings, the results are broken down into a series of seven questions which each have an impact on delineating whether viral load measurements may serve as a suitable surrogate end-point in trials of CMV prevention and treatment.


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 3472) MEDLINE 1342; MEDLINE in-process 100; EMBASE 1839; CDSR 6; Central 141; PubMed (supplemental) 44
Additional records identified through other sources (n =0)
Records after duplicates removed (n =1031)
Records screened (n =2441)
Records excluded (n =2003)
Full-text articles assessed for eligibility (n =438)
Full-text articles excluded, with reasons (n =352)
Studies included in qualitative synthesis (n =86)
Studies included in quantitative synthesis (meta-analysis) (n =45)

Definitions

- CMV disease – refers to symptomatic CMV disease and includes viral syndrome and tissue invasive disease

- CMV viremia- detectable viremia by a quantitative DNA based viral load assay regardless of symptoms
  - A subset of this is asymptomatic viremia

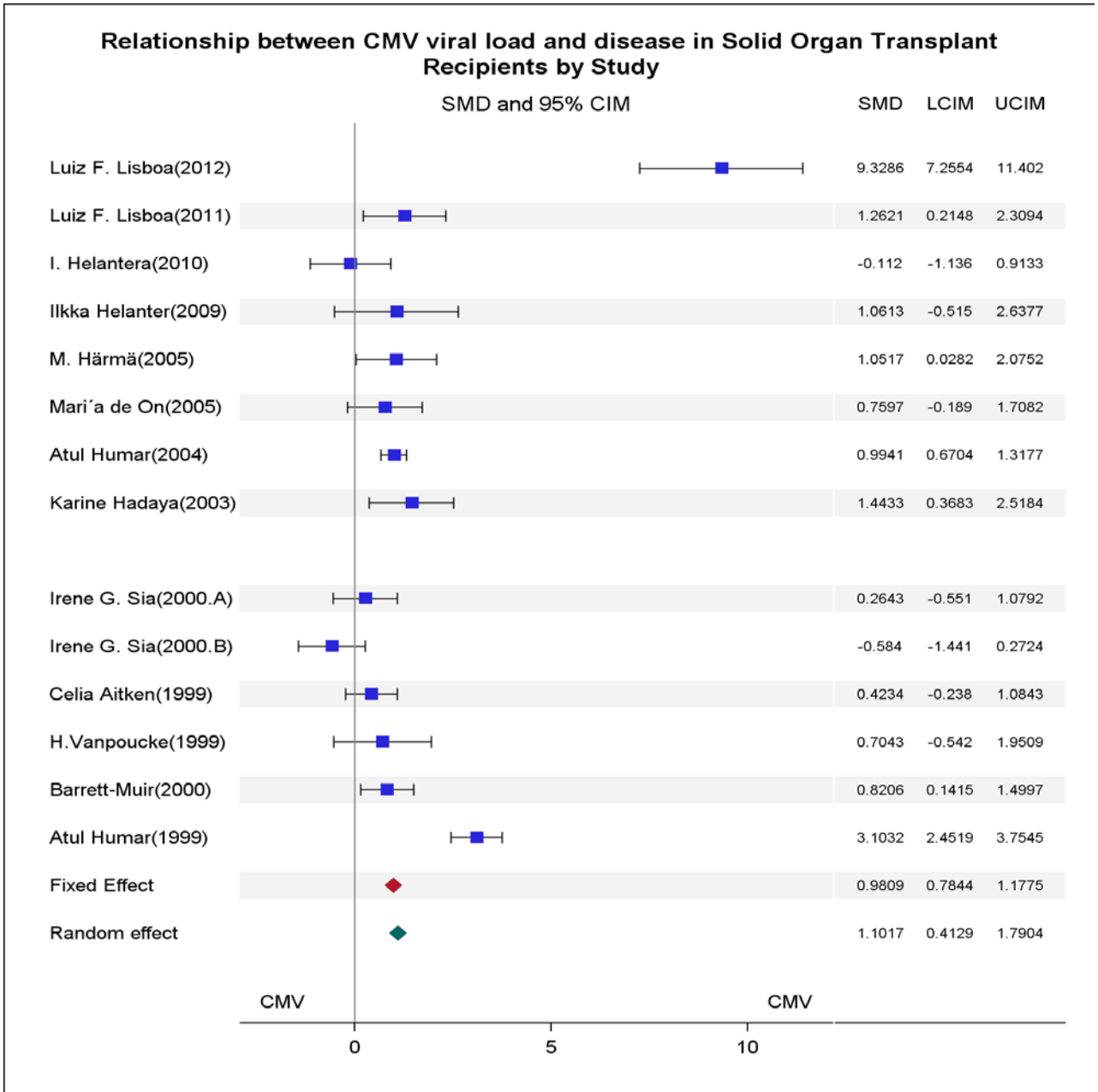
QUESTION 1

Was viral load increased in symptomatic CMV disease vs. asymptomatic viremia?

Question 1

- Forty five studies were identified.[1-45]
- Out of 45 studies, 12 studies were natural history study and the remaining were a combination of prophylaxis and pre-emptive therapy studies
- The most common viral load methodology was the Cobas Amplicor assay.
- Other assays included in-house assays, IU based assays, and others.

Figure 1: results with first 15 studies….



Fixed & Random Effects Model

Model						
	Mean	**SE**	**-95%CI**	**+95%CI**	**z**	**p**
Fixed	0.98093	0.10029	0.78437	1.17750	9.78089	0.00000
Random	1.10165	0.35142	0.41288	1.79042	3.13485	0.00172

Conclusion:

- This metaanalysis shows that in CMV disease, patients have statistically significant higher viral load as compared to the asymptomatic viremia group (p=0.0018 for random effects model).
- This is only with a subset of the identified studies and a complete metaanalysis with all the studies will follow.
- A subanalysis will also be done of just studies using the Cobas amplicor

QUESTION #2
DOES INCREASING VIRAL LOAD CORRELATE WITH INCREASING RISK OF CMV DISEASE?

Does increasing viral load correlate with increasing risk of CMV disease?

- Five studies were identified that specifically examined the change in viral load vs. the risk of developing CMV disease.
- One study showed that the rate of increase in CMV load between the last PCR-negative and first PCR positive sample was significantly faster in patients with CMV disease ($\log_{10}$ 0.33 versus $\log_{10}$ 0·19 genomes/mL daily, $p<0·001$). In multivariate-regression analyses in that study, both initial CMV load and rate of viral load increase were independent risk factors for CMV disease.[1]

Does increasing viral load correlate with increasing risk of CMV disease?

- Another study showed that the degree of viral replication was strongly associated with progression to CMV disease or viremia (risk ratio, 8.8 and 51.5 among patients with virus loads 2860 and 12860 copies/10^6 peripheral blood leukocytes, respectively). [2]
- Other study showed that ten-fold changes in CMV DNA levels at any time point during the first 6 months after transplant predicted for CMV pneumonitis in lung transplant patients. (sensitivity 67%, specificity 93%, $p<0.01$)[3]
- Also there was a 5- to 10-fold increase in the CMV DNA titers prior to disease development.[4]
- An increase in viral load of $\log_{10}0.7$ per week also distinguished between disease and asymptomatic R+ recipients with high sensitivity(100%) and specificity(95%) in kidney transplant.[5]

Does increasing viral load correlate with increasing risk of CMV disease?

- In conclusion, five articles specifically analyzed whether increasing viral load correlates with increased risk of disease. All of these articles suggested that faster viral load increase was correlated with CMV disease.

QUESTION #3
WAS VIRAL LOAD SUPPRESSED ON PROPHYLAXIS?

Was viral load suppressed on prophylaxis?

- In total of 37 prophylaxis studies, we identified 4230 patients. Of these, 24 studies were identified as prophylaxis studies while 13 studies were identified as prophylaxis VS preemptive studies.
- Out of 24 prophylaxis studies, 12 studies documented viral load data in CMV disease and CMV asymptomatic viremia. One additional prophylaxis VS preemptive study documented this data. (Total 13 studies)
- CMV viremia (asymptomatic and symptomatic) was identified in 883/3565(24.8%) (32 studies). Symptomatic CMV disease was identified 517/4204(12.3%) (36 studies).

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Was viral load suppressed on prophylaxis?

- CMV viremia was identified 51/1261 (4.0%) while on prophylaxis. (17 studies)
- CMV disease was identified 35/1634(2.1%) while on prophylaxis. (20 studies)

Was viral load suppressed on prophylaxis?

- **Conclusions**: CMV viremia was identified only 4% during prophylaxis and in total follow-up period, viremia was identified around 25%. In conclusion, there is strong evidence to suggest that CMV viremia was suppressed during prophylactic antivirals and that the incidence of disease was very low during periods of viral suppression (2.1%)

QUESTION #4
DOES DISEASE WHILE ON PROPHYLAXIS CORRELATE WITH DEGREE OF VIRAL LOAD ELEVATION?

Does disease while on prophylaxis correlate with degree of viral load elevation?

- We could not find any study that specifically addressed this issue, or that provided enough detail about viral loads during prophylaxis and their correlation with disease status.
- This was in part because CMV disease was very uncommon during the prophylaxis period.

QUESTION #5
AFTER PROPHYLAXIS DISCONTINUATION, DID THE DEGREE OF VIRAL LOAD ELEVATION CORRELATE WITH DISEASE?

After prophylaxis discontinuation, did the degree of viral load elevation correlate with disease?

- Only 3 studies specifically analyzed this question with enough detail provided to draw conclusions.
- In these three studies, CMV viremia and disease developing after prophylaxis.
- (#8, #15, #21) were specifically evaluated.
- **Conclusion:** consistent with question 1, VL elevation after prophylaxis correlated with disease development although sample size and number of studies were small.

After prophylaxis discontinuation, did the degree of viral load elevation correlate with disease?

	Author	Year	Method	VL in asymptomatic	VL in disease
#8	Luiz F. Lisboa	2011	In house assay	N=6 Median, range 9013(1985-75000)	N=13 Median unknown Range (44200-2666500)
#15	I. Helantera	2010	Cobas Amplicor	N=4 Median19150 Range 400–45700	N=43 Median13 500 Range 1100–283000
#21	Noe´mie Boillat Blanco	2011	In house	N=24 Median unknown Range 330-46100	N=5 Median 5400 Range 330-42600

QUESTION #6
DOES TREATMENT OF ASYMPTOMATIC VIREMIA PREVENT DISEASE?

Does treatment of asymptomatic viremia prevent disease?

- In total 22 studies, we identified 3029 patients who were on preemptive strategy.
- 13 of these studies were identified as prophylaxis VS preemptive studies.
- The incidence of CMV viremia varied from 5.3% (non-mismatch liver transplant) to 92.3% (mismatch kidney, liver and heart). The incidence of CMV disease varied from 0 to 26.3%.

Does treatment of asymptomatic viremia prevent disease?

- CMV viremia was identified in 1105/2970(37.2%). (21 studies)
- CMV disease was identified in 216/2945(7.3%). (20 studies)
- All studies showed lower incidence of CMV disease as compared to CMV viremia.
- In conclusion the rate of CMV viremia was substantially higher (37.2%) than the rate of CMV disease (7.3%).
- This supports the conclusion that treatment of asymptomatic viremia can prevent CMV disease.

QUESTION #7
DOES VIRAL LOAD RESPONSE IN THE TREATMENT PHASE CORRELATE WITH DISEASE (SYMPTOM) RESPONSE?

Does Viral Load response in the treatment phase correlate with symptom response?

- Three studies were identified.
- Two studies were from the VICTOR cohort. One additional study examined the relationship between viral load and pathological resolution.
- The VICTOR study showed a correlation between viral load decrease and symptom resolution in CMV tissue invasive disease and syndrome with both Cobas Amplicor[1] assay and international unit assay[2].

Does Viral Load response in the treatment phase correlate with symptom response?

- Approximately 70% of the patients achieved viral load negativity at Day 49 with Cobas Amplicor.
- Clinical resolution of CMV disease occurred at a mean of 15.1 days.
- Of 267 patients, 251 had CMV disease resolution by day 49 of treatment. Patients with pretreatment CMV DNA of <18 200 IU/mL had faster time to disease resolution (P = .001).
- Patients with CMV load suppression at days 7, 14, and 21 had faster times to clinical disease resolution (P = .005, <.001, and <.001, respectively).

Does Viral Load response in the treatment phase correlate with symptom response?

- There is one other study focusing on biopsy-proven GI CMV disease[3].
- Twenty-six of 274 (9.5%) CMV D+/R− patients developed GI disease. The median half-life of viral load decline during antiviral therapy was 4.7 days.
- Follow-up endoscopy was performed in 20(77%) of the 26 patients. Clearance of CMV from tissue specimens was documented in 17 (85%) of 20 patients.
- Among 17 patients with negative follow-up endoscopy, the median time-to-normal endoscopic finding was 27.0 days after start of induction therapy.

Does Viral Load response in the treatment phase correlate with symptom response?

- In conclusion, even there are only small numbers of studies are identified, viral load response during the treatment phase seemed to correlate with disease response; and specifically achieving viral load negativity correlates strongly with symptom response.

Overall conclusions?

- This systematic review finds evidence to support the validity of viral load as a surrogate marker in trials of CMV post-SOT
- Limitations
  - Different VL assays; few studies with IU/ml
  - Disease definitions not necessarily the same across studies.