TAG AUDIT ON LABORATORY ASPECTS OF VITAMIN D

Audit questionaire additional notes
Dr Laila Tibi

Vitamin D has been implicated in osteoporosis, cancer, high blood pressure, obesity, infertility etc
Labs have seen a rapid increase in work of threefold between 2009 and 2010. There are no NICE clinical, laboratory or reporting guidelines on Vitamin D available. There is antenatal guideline 62 by NICE which is about treatment or prevention rather than measurement.

40-60% of requests are from GPs. 17 labs receive requests from other labs. GOSH has all its requests from the hospital.

50% of labs vet requests. GOSH selectively vet requests. Local guidelines for vetting requests vary between Trusts.

There is no clear difference between the precision of Immunoassay or Tandem MS methods. In the sufficient range the precision is 8% by both methods. In the insufficient range LC MS-MS is 10% and Immunoassay is 14.5 % but in the insufficient range is 4-13 % by both methods.

Immunoassay kits give no calibration or standard details in the kit insert. LC MS-MS use NIST traceable (Chromosystem standard).

The C3 epimer is important in neonatal samples and interferes with some immunoassays and co-elutes in some HPLC method. 6 labs detect this epimer and 4 do not know if their method detects it.

12 labs are in the DEQAS scheme and 1 is trying the Randox scheme.

Reference ranges were harder to define:
Deficient most were <25 or <30 but ranged from <10 to <40
Insufficient most were <50 but ranged from <35 to <80
Replete most were >50 or >75 and ranged from >36 to >80
Toxic levels varied from >150 to >500 (most >200)

Discussion

There were no points of discussion

Laboratory Aspects of Vitamin D. Additional notes
Dr Mandy Donaldson, Imperial

Vitamin D and calcium homeostasis
Data shows that Vitamin D levels peak at the end of the summer and trough in February and March when most of the population is deficient.
Imperial looked at reference interval across the months and found the medium and high levels were 5-10 nmol/L higher in the summer than the winter. There

will be no difference in the low levels. It is thought it is a continuum so it is inappropriate to have seasonal reference ranges.

D3 25OH Cholicalciferol. There is very little from the diet only from fish and milk. It is mainly produced exogenously by sunlight.

D2 Ergocalciferol produced in plants but no real contribution from diet but found in mushrooms. Most comes from over the counter and prescription medication.

1,25 VitD is biologically active but is present in pmol/L quantities. Measurement is useful in parathyroidectomised patients and in renal patients. It is not routinely available as difficult to measure and most requests are not sensible

Vitamin D deficiency

Most renal units put patients on Vitamin D and do not measure levels.

What assay to use?

Specific problems that Vit D is a complex mixture, immunoassays have different specificities. Vit D is difficult to remove from the binding proteins.
LCMS needs investment in new instruments which are laborious, slow and not without interferences
Roche Immunoassay has been on and off the market.
Liason has issues with high workload and is very temperature dependent
Random access assays were slow to come to the market due to the avid binding to binding protein.
LCMS not available on reagent rental, not up to high throughput
Waters has a semi-automated assay with an extraction but takes 24 hours to get a result out.
Some Vacutainers interfere with internal standards

What is normal?

British Endocrine Society 2011
<20 Deficient (causes osteomalacia and needs replacement)
>70 replete
21-69 nmol/L is grey area or insufficient requiring clinical acumen or information to decide on treatment
<50 likely to be consistent with some bone demineralisation
Holick quotes <50 as deficient and 52-72 nmol/L as insufficient.
A lot of the reference range data is from old extraction RIA methods and not from LCMS

Discussion

1. Is your workload from Rheumatology? We see a lot of requests from them but may reflect Consultant speciality within different Trusts.

2. Surprised to see D2 in 50% of GP samples. ? prescription medicine, injections are D2. Over the counter medicine D2 or D3 or some do not say. If you ask in pharmacy do not know.
3. Lots of people taking Vitamin D then go to GP and get Vitamin D measured. Ask if on supplements if do D2 and D3 but prescribed oral therapy can be anything.
4. Roche revised assay at very low levels are lower than referral lab but agree better at the high end. This may be due to the calibrators
5. Was the Abbott work done on the reformulated kit, released in July 2011. This was pre-release work on that kit. On release has been recalibrated to a primary standard, initially it used a secondary standard.
6. IDS values <10 are 10-30 by Abbott but the reference ranges are higher to reflect this.

Clinical Effectiveness Guidelines
Dr Sally Hull from Clinical Effectiveness Group on Vitamin D Guideance in General Practice

Tower Hamlets has 50% ethnic minorities. The Clinical Effectiveness Group supports primary care developments which are adjusted to local need. It took 1 year to draw up the guidelines due to lack of evidence. The aim is to provide evidence based guidelines with pragmatic advice to GPs.
Aching bones, muscles, tired patients are endemic in inner city GP practices. These symptoms relate poorly to Vit D levels. Need to treat patients early so do not miss things.

Diet and Vitamin D

Farmed fish have lower levels than wild fish. In Norway bottles of cod liver oil are on all restaurant tables.
A dark skinned outdoor worker in the Tropics has a Vit D level of 150 nmol/L. Excessive amounts are broken down in the skin. Factor 15 and above block sun exposure and is put on dark skinned children in the playground.

Barts and London Reference Ranges

<30 deficient
30-80 insufficient
81-220 replete/ normal. See optimal PTH and calcium and no bone disease
221-500 high
>500 toxic
It is unclear where the upper level should be.

Oral colecalciferol

100,000 IU gives a rapid response and are back to 60 nmol/L at three months. Most patients start at levels of 6-7 nmol/L so 200,000 units puts them back in the normal range. If 100,000 IU are started early results should stay up.

If you use ergocalciferol orally with the same dose there is less response and levels fall faster. Injections give a slow response and don't get up to the replete range.

Monitoring

8-12 weeks following active treatment with a loading dose. If not high enough give a second loading dose. Monitor calcium levels should be measured at weeks 4 and 8 if risk of hypercalcaemia is high.

Pregnancy and breast feeding

These women need 400 IU/ day as advised in NICE 2008. No monitoring is required for these doses. It may be safe to give 10,000 units to mothers.

Vitamin D products

Only 9% of children aged 1-5 in Tower Hamlets are being prescribed Vitamin drops. This may be in healthy start areas or over the counter. There is variation between different practices.
Calcichew is not popular with patients. There is now a glut of what can be prescribed which range from cheap to costly. Over the counter can be 400, 1000 or 5000 IU colicalciferol.
There are Vit D2 preparations for vegans by DEVA as D2 is made from cod liver/ sheeps hair lanolin.
There are also Halal products which contain no gelatine.

Neurology recommend 10,000 IU Vit D for immune function. It is difficult for GPs to challenge specialise opinions.
There are large price differences for Vit D products but PCT advisors cannot instruct pharmacy to only supply the cheapest

Summary of management guidelines

<30 deficient Osteomalacia, rickets, aches and pain, abdo pain need Vit D replacement by a loading dose followed by supplementation.
31-80 insufficient, associated with disease risk, need supplements, diet and sunshine.
>80, optimal for healthy bones, no treatment required.

Discussion

1. Should we have same blood levels for different skin colours and when do they start getting bone disease. Most studies have been done on white people so this will be difficult to do.
2. Are all vegetarian products suitable for vegans? Solgal D3 is suitable for vegetarians but not vegans.
3. In your practice how many patients have measurable 25OH D2? It is a very small number. Only 1% in some hospitals have measurable D2. Ergocalciferol injections give high levels of D2. What preparations are

prescribed in an area is important as may influence which assay is used.

Is Vitamin D important?

Dr Mark Cohen

Physiology is optimised at a level of 75-100 nmol/L
<50 is deficient
<75 is insufficient
To define deficiency <50 is a good point.
Need to determine which level of Vitamin D normalises PTH.

Classical action of Vit D
Works on nucleus of cell increasing gene transcription
D3 to liver to 1,25 di OH Vit D in the kidney. This increases calcium binding protein which increases dietary absorption.

Non-classical actions of Vitamin D
25OH Vit D in cells converted to 1,25 VitD in cells which is involved in cell signalling
Nephroprotection
Glucose metabolism
Endothelial and cardiovascular protection
Effects on immune system and immunomodulation
Kills TB
Antiproliferation and cellular differentiation in skin cells
Regulation of apoptosis
Control of muscular and neural function

Vitamin D and cancer risk
Calcium and D3 in post-menopausal women reduced 60-70% cancer risk over 4 years. The levels seen were 70-100 nmol/L
Placebo 6.9 % cancer
Calcium and Vit D 2.9 % calcium
Calcium alone 3.8%

Treatment

1000-2000 IU per day will bring Vit D up from 50 to 75 for three month period.
Colecalciferol (Vitamin D3) 20,000 IU per capsule so use 1 per two weeks
If Vit D less than 50 nmol/L a loading dose is needed of 60,000 units weekly for 6-8 weeks which will bring Vit D up to >75 nmol/L
IM Colecalciferol 300,000 IU can be given every three months to bring Vit D up to 75 nmol/L but is costly
Ergocalciferol (Vitamin D2) in BNF is difficult to obtain
Need to give IM if patient will not take tablets
Primary hyperparathyroidism has raised PTH, raised calcium and low Vit D. Need to give Vit D to improve hyperparathyroidism, it may normalise PTH and reduce bone disease.

Patients can get access to high dose Vit D preparations. Adcal used to be only GP prescribable calcium supplement. Need to use cheapest product.

Vitamin D toxicity

Alpha Calcidol is potent and can give toxicity at mg doses.
Vit D2/D3 have a comfortable margin between therapeutic and toxic doses.
10,000 IU per day of Vit D is safe. If take too much Vit D, PTH is suppressed and calcium absorption is reduced. 1,25D has no effect on PTH.
Toxicity is not seen until Vit D >500 nmol/L

Discussion

1. Why do we not use VitD to reduce cancer and cardiovascular risk?
   If <25 or 30 already at risk so need to intervene if less than 50 IU.
   Predisposing factors such as liver/ renal disease aim at 75 or 80
   Evidence that therapeutic vit D is useful? Caution is correct, need to give safe doses for bone health while wait for other trials. Most people working with Vitamin D also take it.

2. Every lab quotes different thresholds and assays also vary so it is difficult to quote universal reference ranges.
   25nmol/L get severe disease and 75-80 nmol/L gives optimal bone health. If above 30 nmo/L are unlikely to be symptomatic bone disease but may have bone disease and is a deficiency state.

3. Children were given cod liver oil in the past, do we give them less Vitamin D today? It contains Vit A which is toxic as well as Vitamin D. Health visitors should see that children are on enough Vit D but parents should ask for it.

Standards and draft guidelines

Draft Laboratory Guidelines for Serum 25 Hydroxyvitamin D (25OH D) Requests

Laboratories should:
Manage the increasing request numbers for serum 25OH D by:

1. Producing Local Guidelines (together with Endocrine, Renal, Rheumatology, Paediatrics, Pharmacy Departments and GPs) as to which patient groups require 25OH D measurements
2. Rejecting 25OH D requests made within 3 months of vitamin D replacement therapy
3. Discouraging routine monitoring in patients on long term maintenance doses

Draft Laboratory Standards for Serum 25 Hydroxyvitamin D (25OH D) Methods

Laboratories should:

1. Use methods with good performance in terms of precision and accuracy particularly at deficient/insufficient 25OH D concentrations
2. Use methods calibrated with standards that are traceable to NIST (National Institute of Standards and Technology)
3. Belong to an EQA Scheme
4. Use chromatographic methods that measure 25OH D2 and 25OH D3 separately
5. Use immunoassays that measure Total 25OH D using antibodies with an equimolar response to 25OH D2 and 25OH D36.
6. Use methods that can distinguish and/or show no cross reactivity with C-3 epimers of 25OH D2 and 25OH D3 (especially when the laboratory processes paediatric samples)

Draft Laboratory Standards for Serum 25 Hydroxyvitamin D (25OH D) Results

Laboratories should:

1. Report Total 25OH D concentrations (by addition of 25OH D2 and 25OH D3 for chromatographic methods)
2. Report 25OH D results within 2 weeks of receipt of sample
3. Whenever possible use Serum 25OH D "consensus" reference ranges
    a. less than 25 nmol/L: profound vitamin D **deficiency** (symptomatic osteomalacia or rickets)
    b. between 25 and 50 nmol/L: vitamin D **insufficiency** (associated with several common diseases including cardiovascular disease, diabetes, cancer, multiple sclerosis)
    c. greater than 500 nmol/L: associated with Vitamin D **toxicity**

Pearce SHS, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ 2010; 340: 142 - 147

Discussion

Standard 1 Requesting

Producing Local Guidelines (together with Endocrine, Renal, Rheumatology, and Pharmacy Departments) as to which patient groups require 25OH D measurements

Add in Paediatricians and GPs.

When producing local guidelines labs can vet out requests from sources which should not use it. This is not practical so can only do it with a time limit. There does need to be agreement with the clinicians. New commissioning consortia are interested in the costs of both tests and medication. Expensive test and expensive medication so need to be seen as cost effective. Vitamin D treatment costs £36 per year for 1000 units /day but can pay £95 for 5 capsules.

Vitamin D is topical so can work together with them to agree guidelines. What are effective forms of communication between Pathology and GPs? Results sent out with comment added. Education of individuals or whole GP body. Pathologists alongside physicians talking about Vitamin D

Standard 2 Requesting

Rejecting 25OH D requests made within 3 months of vitamin D replacement therapy

This should be done by educating GPs but to save money need to do electronically at the requesting stage. This is difficult to do practically. Need to be able to over-ride the decision so are all vetted by Duty Biochemist.

Standard 3 Requesting

Discouraging routine monitoring in patients on long term maintenance doses.

This is difficult to put into practice. When 1 lab stopped GPs requesting B12 in patients on maintence doses the number of requests went up.

Standard 1 Methods

Use methods with good performance in terms of precision and accuracy particularly at deficient/insufficient 25OH D concentrations.

Do we need a figure as is variable on QC scheme? It is not possible to set a figure for precision and accuracy. Labs should achieve what is in the kit insert.

Standard 2 Methods

Use methods calibrated with standards that are traceable to NIST (National Institute of Standards and Technology).

We need more standardisation all round.

Standard 3 Methods

Belong to an EQA Scheme.

There are currently DEQAS and Randox

Standard 4 Methods

Use chromatographic methods that measure 25OH D2 and 25OH D3 separately

This should be changed to if using chromatographic methods measure D2 and D3 separately

Standard 5 Methods

Use immunoassays that measure Total 25OH D using antibodies with an equimolar response to 25OH D2 and 25OH D36. Use methods that can distinguish and/or show no cross reactivity with C-3 epimers of 25OH D2 and 25OH D3 (especially when the laboratory processes paediatric samples. Should this say need to know the antibody response to D2 and D3 in the method used. C3 epimers in Paediatric samples are present in the neonatal period up to age 1 month.

Results

Standard 1 Results

Report Total 25OH D concentrations (by addition of 25OH D2 and 25OH D3 for chromatographic methods)

Standard 2 Results

Report 25OH D results within 2 weeks of receipt of sample

Standard 3 Results

Whenever possible use Serum 25OH D "consensus" reference ranges

less than 25 nmol/L: profound vitamin D ***deficiency***
(symptomatic osteomalacia or rickets)

between 25 and 50 nmol/L: vitamin D ***insufficiency***
(associated with several common diseases including cardiovascular disease, diabetes, cancer, multiple sclerosis)

greater than 500 nmol/L: associated with Vitamin D ***toxicity***

Are Pathology Harmony looking at these?
Upper boundary >50 should be higher than where lower boundary lies. 75-80 nmol/L have a normal PTH with maximal absorption of calcium and optimal bone health
75-80 is optimal level 25-30 is deficient
Add a replete range. Optimal is 75-200 or 80-220 nmol/L
25 nmol/L is severe deficiency
There is lack of standardisation between methods, assays do not agree.
DEQAS samples are mostly high, not very low levels. Collected from patients for venosection and they have a limited range of patients. They are true patient samples and not a spiked pool.
We could say between 25 and 75 nmol/L depending on the assay. Need to be aware of assay properties.
We seem to have agreed on toxicity and deficiency.

Consensus reference ranges from all the presentations

Deficient

Range mol/L	Source
<20	British Endocrine Soc
<25	TAG mode
<30	Royal London
<50	Mark Cohen
<50	Holick

Insufficient

Range mol/L	Source
21-69	British Endocrine Soc
30-79	Royal London
30-80	Barts
<50	TAG mode
51-74	Mark Cohen
52-72	Holick

Replete

Range mol/L	Source
>50	TAG mode
>70	British Endocrine Soc
>80	Mark Cohen
80-150	Royal London
81-220	Barts

High

221-500 nmol/L Barts

Toxic

>500 nmol/L Royal London