Vitamin D and Pancreatic Cancer Risk – No U-Shaped Curve

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Abstract. *In a recent paper entitled "Circulating 25-Hydroxyvitamin D and the Risk of Pancreatic Cancer," Stolzenberg-Solomon et al. reported that the odds ratio for diagnosis of pancreatic cancer shows a "statistically significant" precipitous jump of more than a factor of 2 at the highest presented concentrations of >100 nmol/l. This was one of six related studies of the relation of 25-hydroxyvitamin D and six types of rarer cancers, collected by the Vitamin D Pooling Project (VDPP). An alternative analysis of the presented data suggests that the reported two-fold higher risk at the highest serum 25(OH)D level in the pancreatic cancer study is most likely a statistical artifact associated with the chosen cut-off point groupings and there is no U-shaped curve to be explained.*

In their recent paper "Circulating 25-Hydroxyvitamin D and the Risk of Pancreatic Cancer,"(1) Stolzenberg-Solomon *et al.* have presented an analysis of odds ratios of diagnosis of pancreatic cancer as a function of the serum level of 25 hydroxyvitamin D. As reported, the odds ratio was nearly constant at approximately 1.0 in all groups the one below with the highest concentrations >100 nmol/l, where the indicated odds ratio more than doubled. This is, indeed, rather striking. This result has been widely cited as a cause for concern. In the abstract the authors wrote: "...a high 25(OH)D concentration (≥100 nmol/l) was associated with a *statistically significant* 2-fold increase in pancreatic cancer risk overall (odds ratio=2.12, 95% confidence interval: 1.23, 3.64). Given this result, recommendations to increase vitamin D concentrations in healthy persons for the prevention of cancer should be carefully considered".

This result was deemed important enough to be cited in the recent Institute of Medicine Report on Dietary Reference Intakes for Calcium and Vitamin D (2): "Byers (3), in a recent editorial commenting on the outcomes of a pooling study focused on vitamin D and six types of cancer in which the *only association* observed was a *doubling of the risk* for pancreatic cancer for those in the highest *quintile* [*sic*] of circulating serum 25OHD levels, offered the following observation: "We have learned some hard lessons.... and *we now know* that taking vitamins in supernutritional doses can cause serious harm".

The aim of this article is to examine the evidence presented, and to judge its statistical significance.

The Pattern

The computed odds ratios are collected in Table 3 in the article of Stolzenburg-Solomon *et al.* (1) and reproduced here in Table I.

Two striking features stand out in Table I: (i) The differences in the ranges of cut-off points for the groups (two groups spanning 12.5 nmol/l, two other groups spanning twice that at 25 nmol/l and the end groups – those below 25 and those above 100 nmol/l), and (ii) The wide variation in the populations of these groups. The number in the highest group (>100 nmol/l) is 3.2% of the total, while the number in the reference group (50-<75 nmol/l) is 33.4%, or more than 10 times as large.

The "statistically significant 2-fold increased risk" is readily apparent (see Figure 1).

Biological Plausibility

The pattern exhibited here is truly astonishing. The concentration-related response to serum concentrations of a micronutrient such as 25(OH)D is typically a monotonic trend. A flat trend line followed by a very steep inflection is possible, but rarely seen. The known toxic level for 25(OH)D is around 500 nmol/l, five times the level at which this sudden inflection appears (4).

Chosen Cut-off points and Resulting Groups

As is emphasized in many textbooks, the particular choice of cut-off points which divide the data into groups can have a substantial effect on the pattern that emerges (5). Analysts

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Key Words: Statistical methods, vitamin D, pancreatic cancer risk.

Table I. *Odds ratios for the association between circulating 25-hydroxyvitamin D concentrations and risk of pancreatic cancer [extracted from Stolzenburg-Solomon et al. (1) Table 3].*

Group	Serum 25(OH)D Level nmol/l	Cases	Controls	Total	OR*	OR**
1	<25	115	141	256	0.98	1.22
2	25-<37.5	164	225	389	0.97	1.09
3	37.5-<50	208	286	494	1.06	1.09
4 ref	50-<75	306	458	764	1	1
5	75-<100	120	190	310	1.01	0.95
6	≥100	39	33†	72	2.05	1.77

*"Crude" line, "adjusted for matching variables" (note d in their table). **ORs calculated from the listed Cases and Controls, using web available calculator (10). †In the Table 3 that appears in (1), in the highest group it shows the number of cases/controls as 39/30. Adding across the six groups gives a total of 2282 participants, with 952 cases and 1330 controls, while their footnote "a" indicates 952 cases and 1333 controls. Comparison with their Table 4 shows a total number of controls in the highest group to be 33, not 30. It is assumed that their computations were made using 33 controls, and that the notation in Table 3 is a typographical error. Table I in this paper reflects this correction.

Table II. *Derived from Table I by combining groups 5 and 6 above into group 5*.*

Group	Serum 25(OH)D Level nmol/l	Cases	Controls	Total	OR**
1	<25	115	141	256	1.22
2	25-<37.5	164	225	389	1.09
3	37.5-<50	208	286	494	1.09
4 ref	50-<75	306	458	764	1
5*	≥75	159	223	382	1.07

often try several different selections of cut-off points. Patterns that remain highly similar are deemed "robust", while those that appear uniquely with particular groupings arise curiosity, and sometimes lead to very interesting further investigations, but usually carry less weight.

While not universal, the most common analytical choice is to form groups of (nearly) equal size, such as quartiles, quintiles, etc. The cut-off points shown in Table I were not chosen in the original article to give six groups of equal size, but instead were chosen to be "clinically relevant". They mark the serum levels associated with widely used qualitative descriptions of "deficient," "insufficient," "sufficient". While this serves the purpose of relating the cut-off points to familiar clinical levels, it has the consequence of generating groups of widely varying sizes – including one substantially smaller than the others. This, smallest group, also happens to be the one of greatest interest.

What happens if the data for the smallest group with the highest concentrations are combined with those of the next lower group? This alternate grouping was used in some of the tables in parts of the Vitamin D Pooling Project of Rarer Cancers (VDPP) report. As can be seen by comparing Tables I and II, this still leaves the resulting new Group 5* as the *second* smallest group.

The odds ratios shown in the next to last column of Table I [taken from (1)], have been "adjusted for matching variables". Not having the source data, we make use of the numbers of cases and controls shown in the table to compute a set of *unadjusted* odds ratios. These are shown in the last column of Table I. Figure 2 illustrates the influence of the adjustment. As can be seen, this does not materially change the most salient feature – a sudden increase in the highest group.

On the other hand, when the two highest groups, 5 and 6, are combined (see Table II) this sudden jump simply disappears - compare Figure 2 with Figure 3. It seems unlikely that this disappearance would be significantly changed by the "adjustment for matching variables".

The clear difference in qualitative pattern, shown by the regrouping of 3% of the data would indicate that the sudden two-fold rise and the conclusions drawn from that pattern is not robust, and could be misleading for some readers of (1).

Relation to the Other Studies in the VDPP

The paper by Stolzenberg-Solomon *et al.* (1) is one of a collection of six individual studies, together with a three overview papers, that make up the report of the VDPP, published together in the American Journal of Epidemiology (6). These papers reported the outcomes of six related studies, with a common format, relating the measurement of the serum level of vitamin D in archived blood samples to later diagnoses of six types of cancer. These included kidney, non-Hodgkin lymphoma, upper gastrointestinal, pancreatic, endometrial and ovarian. The overall results are summarized in an "Overview" paper by Helzlsouer (8). *No association* was found in five of these studies. The *only* association of note was for pancreatic cancer, with its curious doubling of the odds ratio at the highest serum level.

The "association" under investigation was to test whether the diagnosis of the relevant cancer was associated with serum 25(OH)D from a single sample of blood collected years before the diagnosis. The interval between the collection of the single sample and the time of diagnosis, the "lag time," was an average of ~6.5 years (interquartile range 2.8 to 10.9 years). The full range was from near zero to approximeately 30 years.

References are cited in the article to studies of temporal stability of small groups of participants (30, 71, 144 in the original article) over periods of 2 to 3 years. None of the

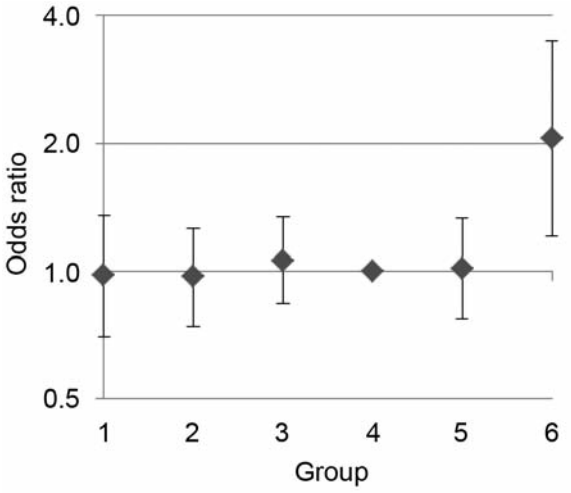

Figure 1. *Odds ratios with 95% confidence intervals [extracted from (1)].*

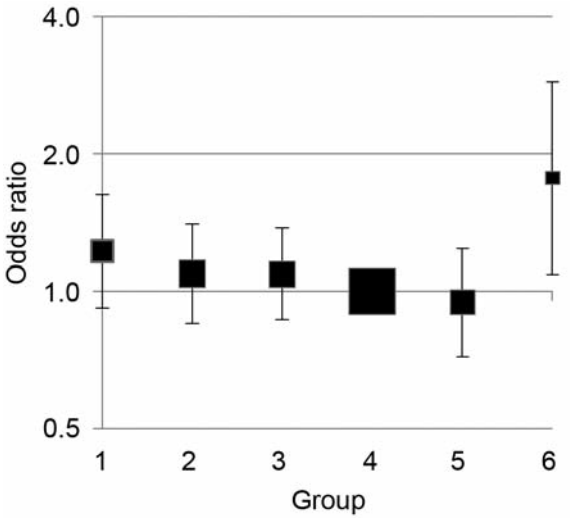

Figure 2. *Odds ratios, using unadjusted numbers (see Table I) Areas of symbols are proportional to the numbers in each group.*

cited studies deals with time correlations over periods of 6 to 30 years. A review by Grant (9) following risk ratios for three types of cancer as a function of follow-up period, concludes that the results "are consistent with a diminishing utility of one-time serum 25(OH)D measurements for determining the effect of vitamin D in reducing the risk of cancers as the follow-up time increases".

The VDPP results provide some relevant data. There are 36 data points, the ratios of cases to controls for six parallel studies as six serum levels each. Six of these are chosen as the reference ratios. This leaves 30 points of variation. The question to be posed is how many of these should be expected to lie within the 95th percentile range of their references, and how many would be expected, entirely by chance, to lie outside that confidence limit?

With 30 "random" samples, each having a probability of 2.5% of lying *above* the reference (along with 2.5% of lying *below* the referent – which would not be of great concern here), the "expected average" would be 0.75. Then the probabilities of observing *no* such outlier would be 0.47, while the probability of observing seeing one or more would be 0.53. Therefore, the finding that one point in this collection of measurements is statistically significant is entirely consistent with the null hypothesis. There is no "statistically significant" increase to be explained.

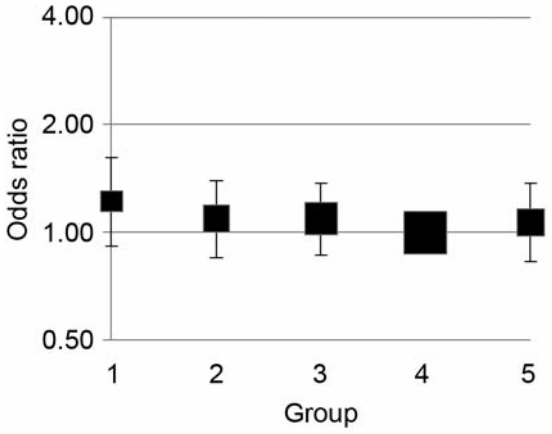

Figure 3. *Odds ratios when the two highest groups are combined – (see Table II) Areas of symbols are proportional to the numbers in each group.*

Discussion

In their article, Stolzenberg-Solomon *et al*. conclude that "…Before any conclusions regarding vitamin D's potential role(s) in the etiology of pancreatic cancer can be reached, more research is required, including prospective studies and laboratory investigations of biologically plausible mechanisms that may explain the observations. …"

We echo the need for more research. Two directions for future investigations that would prove fruitful would include first finding, or generating, cohorts with significantly higher serum levels – with the highest quantiles above 150 nmol/L, and second supporting as standard of care the routine measurement of the 25(OH)D serum level at the time of diagnosis for any serious disease, such as cancer. This would go a long way to removing the necessity of finding a surrogate measure for this critical independent variable, especially one removed by many years from a diagnosis of cancer.

As an interim step, an explanation of the observations might be found in a careful re-examination of the data already available. Specifically, an exhibition of the result of choosing cut-off points that generate 6 (or 8, or 10) groups of

nearly equal size could demonstrate the robustness of the suggested trend. This can, of course, only be done with the original data sets. It would be of great interest if such groupings were exhibited to see if the resulting patterns change with a change in cut-off points.

We do not underestimate the effort required to produce two or three new tables of odds ratios. On the other hand, it is something that can be done with existing data, and while substantial, such an effort would be small compared with the effort that has been expended in assembling the data.

In light of the evidence so far made available to scientists, we conclude that a U-shaped curve for pancreatic cancer risk as a function of 25(OH)D serum level has not been convincingly demonstrated.

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Received December 22, 2011
Revised February 9, 2012
Accepted February 13, 2012